

# The Anatomy and Functional Morphology of the Reproductive System in the Opisthobranch Mollusk

*Phyllaplysia taylori* DALL, 1900

BY

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(5 Plates; 13 Text figures)

## INTRODUCTION

HERMAPHRODITISM EVIDENTLY IS one of the basic features of the opisthobranch mollusks. As might be expected in such a large, diverse, and morphologically flexible group, an immense array of anatomical patterns seems to have arisen to cope with the many functional problems involved where both sex roles are carried out simultaneously in the same individual.

Some information on these systems was included in the large opisthobranch literature which had developed by the early part of the twentieth century, but the total picture of opisthobranch reproduction was very confusing. Most of these older papers treated the reproductive system only from a taxonomic or strictly anatomical viewpoint. Considerations of function were often very limited or were based on speculation rather than on critical observation and experimentation. GHISELIN (1964, 1965) rendered an invaluable service by drawing together information on opisthobranch reproduction from both older and newer literature in an attempt to logically reconstruct the phylogeny of the opisthobranchs. The variety and complexity of the reproductive system provided a pivot point for that study; thus, probable homologies and functions of reproductive structures were stressed. That survey points out the need for much additional study of form and function within specific groups.

The scarcity of functional information in the older papers is due only in part to the rarity of a functional anatomy perspective. It was also due to a lack of techniques for satisfactorily studying living processes. In 1957 the technique of labeling newly replicated deoxyribonucleic acid by tritiated thymidine was introduced (TAYLOR *et al.*, 1957). The site of origin and the subsequent move-

ments of cells so labeled can be detected by autoradiography. Phase-contrast microscopy also provides a valuable modern tool for observing living material. Electron microscopy adds new dimensions to the study of fixed material. In the present study such new techniques have been applied to old unanswered questions of opisthobranch reproductive function.

The order Anaspidea (the "sea-hares") (defined by BEEMAN, 1968a) is especially interesting for its complex, incompletely divided reproductive tracts which are probably close to the ancestral patterns of its clade. Despite several papers relating to reproduction in this group, many gaps and conflicts remained. The early accounts, sometimes colorful and often grossly inaccurate, of PLINY (60 A.D.), CUVIER (1803), and others have been reviewed by MAZZARELLI (1891, 1893a), EALES (1921), WINKLER (1957), and LINTON (1966). Most of the study of anaspidean reproduction has been concerned with *Aplysia*, the large sea-hare. Such study had its serious start with the masterful work of MAZZARELLI (1891, 1893a). The memoir on *Aplysia* by EALES (1921) is a key work of the early twentieth century. LLOYD (1952) presented one of the few good studies of opisthobranch reproductive anatomy, but her treatment of a single anaspidean (*Aplysia punctata* CUVIER, 1803) was superficial; many of her comments were based on mistaken notions of gamete routing. WINKLER (1957) added limited, primarily speculative, information on reproduction in *A. californica* COOPER, 1863. Hopefully, my recent studies of aplysid reproductive biology (BEEMAN, 1966, 1968a, 1970a, 1970b, 1970c) have helped to resolve many of the key questions concerning the reproduction of these animals. THOMPSON & BEBBINGTON's (1969) report on reproductive anatomy and function in *Aplysia* independently added

support and details to some of these studies and provided the first transmission electron microscopy of the mature aplysid sperm.

The present paper is a modification and expansion of my previous work on reproductive structure and function (BEEMAN, 1966). This study is basically an attempt to elucidate the reproductive anatomy and functional morphology of the anaspidean *Phyllaplysia taylori*. This is a small, bright green, striped sea-hare (Figures 1 and 2) abundant on the marine angiosperm *Zostera marina* LINNAEUS, 1758 in the bays and estuaries of the north-eastern Pacific Ocean. There has been relatively little known about it or any other member of the sub-family Dolabriferinae.

Only McCAULEY (1960), MARCUS (1961), and MACFARLAND (1966) have provided any information, previous to my studies, on the reproductive system of *Phyllaplysia taylori*. These reports are very limited and contain severe conflicts. MACFARLAND'S (1966) post-humous memoir contains the most accurate work ever published on the reproductive system of this species, but its coverage of individual organs is brief and many sections were obviously never completed for the published manuscript. It contains no information on function.

The present report is divided into two parts, one on the anatomy and histology, and another on the functional morphology, each with its own methods and materials section.

## I. ANATOMY AND HISTOLOGY OF THE REPRODUCTIVE SYSTEM

### METHODS AND MATERIALS

Animals for anatomical study were taken directly from Elkhorn Slough, Monterey County, California (36°48' N; 121°47'15" W) or from populations obtained at the slough and maintained in large cement tanks in a greenhouse or out-of-doors at Hopkins Marine Station, Pacific Grove, California. These tanks, held at 14° - 16° C by running seawater, provided very favorable conditions, including rich growths of sessile diatoms for food.

Much of the anatomical study was done on fresh or relaxed live specimens. Most were relaxed by a one-hour immersion in a magnesium chloride solution isotonic with seawater. The succinylcholine relaxation procedure of BEEMAN (1968a, 1968b) was occasionally used for extremely rapid relaxation; this method was especially useful in the functional morphology study to observe internal functions in progress.

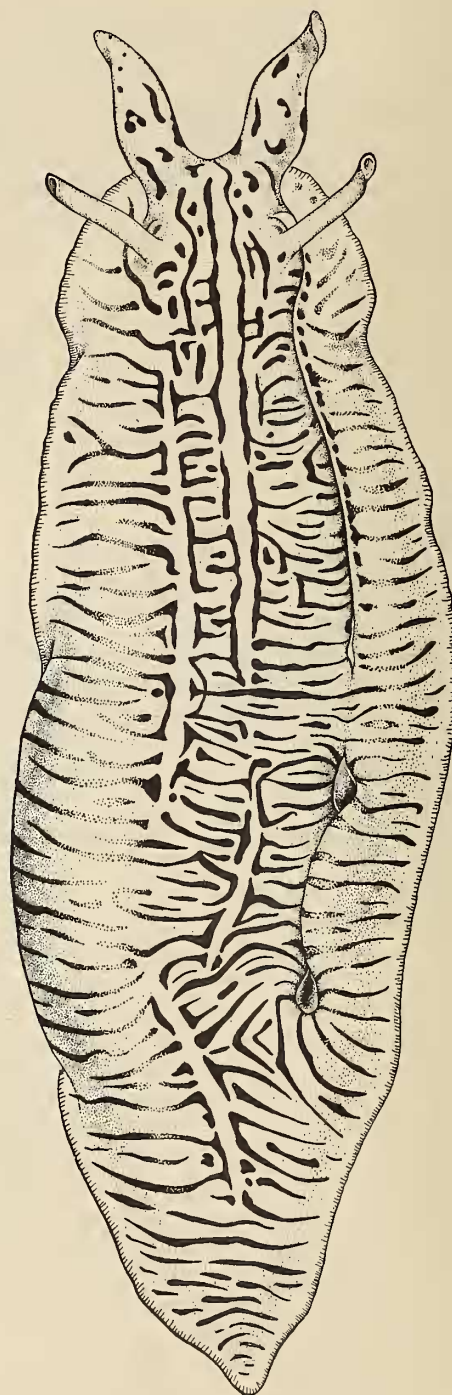


Figure 1

*Phyllaplysia taylori*

Dorsal aspect of a 4.5 cm individual, from life  
(from BEEMAN, 1968a)

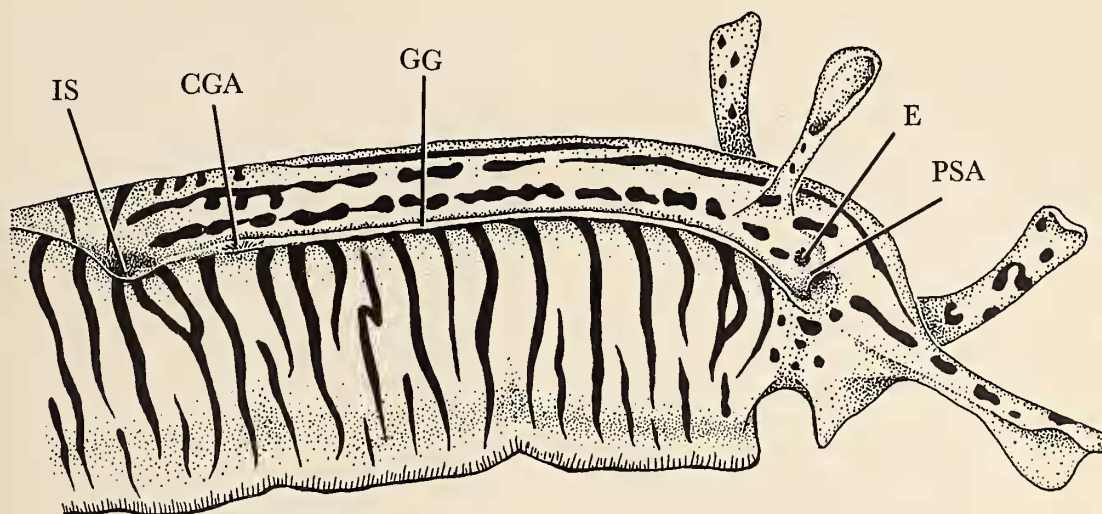


Figure 2

*Phyllaphysia taylora*

Right lateral aspect of a 4.5 cm individual, from life. The labial lappet is directly ventrad from the penial sheath aperture (Modified from BEEMAN, 1968a)

Note: Legend abbreviations for all figures are on the foldout near the end of this article.

Methyl green stain was extremely useful in the dissection work. This stain, originally suggested for *in toto* staining of nudibranch mucous glands (RACOVITZA after LEE, 1928), colors just the mucoid secretory areas and is thus especially useful in contrasting parts of the female gland mass. Methyl green worked best on material fixed in ethyl alcohol or seawater Bouin's solution. Fixed animals were transferred to 70% ethyl alcohol and allowed to remain overnight after the addition of several drops of a stock solution of 1% methyl green in 50% ethyl alcohol.

Animals to be fixed were given a rapid intra-hemocoelic injection of seawater Bouin's fixative into the anterior left quarter of the animal. This afforded almost instant fixation of the nervous system and muscles, thus eliminating most contraction artifact and providing minimal disturbance which might affect the position of gametes. Animals thus killed were fixed overnight in seawater Bouin's solution under refrigeration.

Paraffin sections were cut at  $2\mu$  to  $20\mu$ ; most examinations were made on  $7\mu$  slices. Most non-autoradiographic specimens were stained in Kessel's modification of Mayer's Haemalum (Clifford Grobstein, pers. comm., 1962) and counterstained in Galigher's Triosin. Several preparations were stained in Mallory's Triple Stain or Mallory Heidenhain's Azan Stain (HUMASON, 1962). Autoradio-

grams (discussed in the functional morphology section) stained with both Mayer's Haemalum and Celestine Blue B, without any counterstaining, were especially useful for examination of many cellular features. The periodic acid-Schiff reaction (PAS) was used to demonstrate 1:2 glycol groups or equivalent amino or alkylamino derivatives (MOWRY, 1963). Complete or partial serial sections were prepared of 240 animals, ranging in fixed weight from 0.1 mg to 15.1 g.

Material for scanning electron microscopy was fixed in 70% ethyl alcohol, dehydrated through an ethanol series, and cemented to aluminum disks. These disks were vapor coated with gold on a rotary shadowing stage in a Varian vacuum evaporator, affixed to specimen stubs with silver paint, and examined in a Cambridge Stereoscan scanning electron microscope.

## TERMINOLOGY AND GENERAL FEATURES

The terminology used in this study mainly follows that of GHISELIN's 1965 comparative study of opisthobranch reproductive systems. It should be noted, however, that GHISELIN uses structural names, not in reference to function, but to inferred homologies. Some terms have been

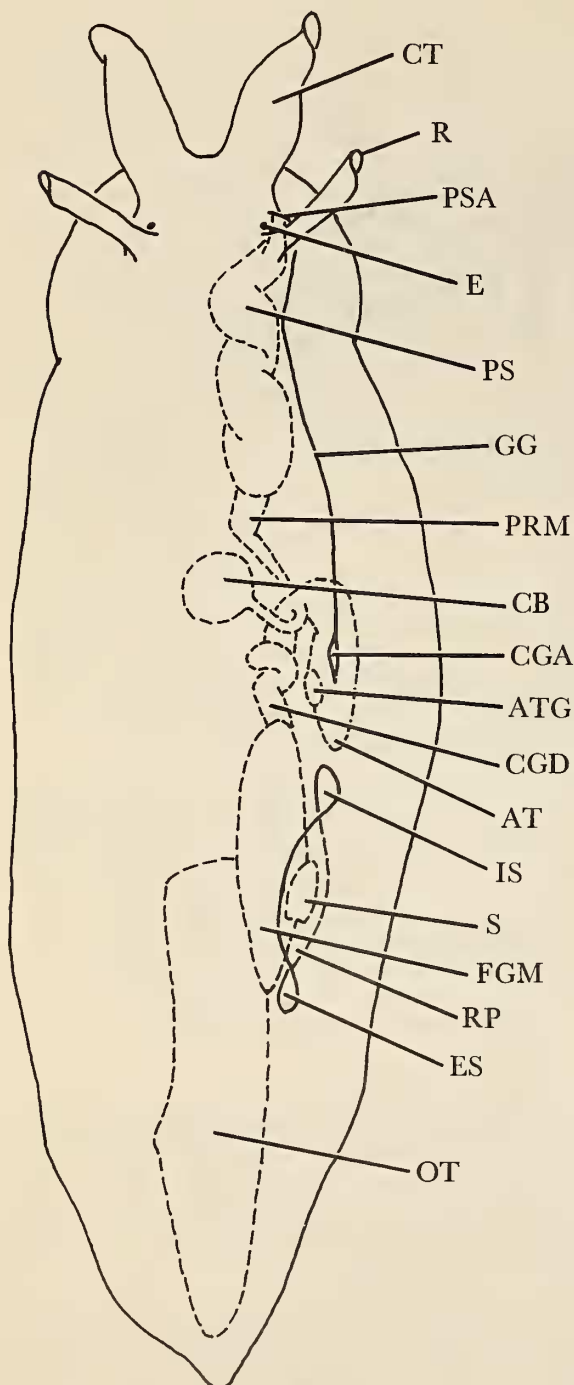


Figure 3

*Phyllaplysia taylori*

Semi-diagrammatic outline indicating main external features and reproductive system

(Modified from BEEMAN, 1968a)

anglicized for the sake of consistency. The "external seminal groove" of his study is here called the external genital groove since it carries both semen and ova. There is some question that his choice of "membrane gland" is a happy substitute for what is often referred to as the "covering gland" by prosobranch workers (FRETTER & GRAHAM, 1962, etc.). However, it is a far better term than "winding gland," an inaccurate term without functional or comparative value which has been used regularly by students of anaspideans. "Ampulla" is retained as a name for a structure which is almost certainly homologous to the ampulla or seminal vesicle of male prosobranchs and which is represented in female prosobranchs only by a corresponding but unmodified segment of the gonoduct. Ampulla is a term well known to opisthobranch workers and is an improvement over seminal vesicle considering that the structure here has a female as well as a male role. I have also followed the concept, used by GHISELIN and others, of dividing the gonoduct into pallial and coelomic regions. This concept seems to be supported by THOMPSON's 1962 study of nudibranch ontogeny, if ectodermal origin is accepted as evidence of pallial origin.

Comparing the terminology used here to that of other authors, one finds the situation has not changed completely since BRUEL (1904) wrote of the contradictions and tangles which were involved in the naming of various vesicles. GHISELIN's work has helped to clarify terminology for the reproductive systems of opisthobranchs as a group, and the present work gives synonyms at several points where possible confusion may arise in the anaspideans. Further discussions of terminology, old and new, are available in MAZZARELLI (1891) and PRUVOT-FOL (1960).

The general association of the reproductive organs of *Phyllaplysia taylori* is shown in Figures 3, 4, and 5. Starting posteriorly, a large ovotestis empties into the coelomic gonoduct which consists of the ampulla and its pre- and post-ampullar ducts. The post-ampullar duct leads to the fertilization chamber, focal point of the female gland mass and beginning of the pallial gonoduct. The female gland mass is composed of the albumen, membrane, and mucous glands. The pallial gonoduct consists of the common genital duct and its associated organs. The common genital duct connects the above 3 pallial glands to the outside. This outgoing tube is incompletely divided into 2 ducts, the pallial spermoviduct and the copulatory duct. Three chambers open into the common genital duct: the seminal receptacle, the copulatory bursa, and the atrium. The common genital duct opens externally as the common genital aperture. The external genital groove runs forward along the right external body surface from this aperture to the penis ensheathed within the right side of the head.

## OVOTESTIS

Most references to the dolabriferean ovotestis have been made in connection with taxonomic studies; authors have especially noted whether the organ was lobate or non-lobate, a feature which probably depends to some degree on the state of preservation and the extent of dissection. The term "ovotestis" is preferred to its synonym, "hermaphroditic gland," as there is no evidence of glandular secretion. LÜSIS (1961) suggests that development of most of the hermaphroditic reproductive system in the pulmonate *Arion* is independent of the ovotestis, as development of other reproductive structures proceeds normally even if growth of the ovotestis is retarded. Other reports, also from outside the opisthobranchs, give conflicting conclusions from meager evidence. LAVIOLETTE, in a very short 1956 review, states that there is some evidence of hormonal control by the gastropod gonad.

In mature specimens the ovotestis is the largest of the reproductive organs and second largest organ in the body, being exceeded only by the digestive gland. It is roughly cone-shaped and extends for about  $\frac{1}{3}$  of the body length, from a narrow posterior tip which fills the rear of the hemocoel to a blunt anterior end (Figure 3). Anteriorly, the left side is strongly indented by the rounded surface of the digestive gland.

The lobate nature of the ovotestis is made visible upon slightly spreading the organ (Figure 4). The numerous lobes are completely independent, being bound together only by a covering membrane and by the converging branches of the pre-ampullar duct. The branches join to form the main pre-ampullar duct which leads forward from the ovotestis to become the ampulla.

The color of the ovotestis is visible through the ventral surface of the living animal. The organ is quite greenish in most specimens, greenish-yellow in animals with large oocytes, greenish-yellow with yellow spots when oocytes are ripe, and white (and shrunken) in starved individuals. The thin membrane which encloses it often has longitudinal brown stripes of a color similar to that on the exterior of the animal.

Except for the simple squamous epithelia which ensheath the individual lobes and the entire organ, almost every cell of the ovotestis appears generative. A definite germinal epithelium does not exist; both sperm and ova in the last stages of gametogenesis could be found attached directly to the simple bounding membrane, while gametocytes of similar, less, or no visible differentiation form groups along this membrane and within the lobe. This observation on the lack of a germinal epithelium agrees with that of MAZZARELLI (1891) for *Aplysia*, but his finding that all the gametocytes differentiate at the

same time and thus provide only one breeding season does not agree with the situation in *Phyllaplysia taylori*. There appears to be a decreasing reserve of undifferentiated gametocytes throughout the life of the animal.

Each lobe of the ovotestis is composed of numerous acini, each acinus simultaneously containing a mixture of all stages of male and female cells after the onset of sexual maturation (at about 20 mg body weight). The oocytes are somewhat more common on the periphery, but this tendency is by no means as well developed as in my sections of *Aplysia californica* ovotestis where the great majority of the oocytes is found toward the outside. The lumen of each acinus is filled with the tails of developing sperm, finished gametes, cell fragments, and amebocyte-like cells.

Very early oocytes and spermatocytes could not be distinguished from one another. Oogenesis as such was not studied, though measurements of oocyte diameter were made in connection with growth studies. No division of oocytes was observed in the ovotestis. Oocyte yolk granules show a strongly positive PAS reaction. Details of spermatogenesis in *Phyllaplysia taylori* were presented by BEEMAN (1970a). Oogenesis was nicely reported for *Aplysia* by THOMPSON & BEBBINGTON (1969).

## COELOMIC GONODUCT

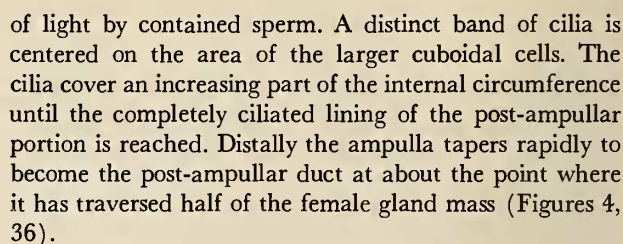
The coelomic gonoduct, referred to by some authors as the ampulla or little hermaphroditic duct, is a single tube leading from the ovotestis to the fertilization chamber. GHISELIN'S (1964) convenient subdivision of the duct into 3 regions, a pre-ampullar portion, an ampulla proper, and a post-ampullar portion, is accepted for the present work.

## 1. Pre-ampullar Portion (PRA)

The pre-ampullar portion consists of tiny tubules which converge from the individual lobes of the ovotestis and unite into a single, larger, relatively straight tube which extends anteriorly to about the anterior end of the ovotestis (Figure 4). The thin wall of this tube is little more than a squamous or cuboidal epithelium. The forward end dilates to become the ampulla proper.

## 2. Ampulla Proper (AM)

The main body of the ampulla is a large, highly convoluted tube (Figure 4). Cuboidal cells, slightly larger on one side of the tube, form a lining externally bounded by sparse connective tissue or muscle fibers and a very thin squamous layer. The tube is generally distended by sperm and oocytes or separate masses of each. The ampulla has a glistening pearly appearance which is due to reflection



### 3. Post-Ampullar Portion (POA)

The post-ampullar portion or duct leads forward from the ampulla, runs along the left face of the female gland mass, moves over its forward edge to the right face, arches up and over the ventral lobe of the albumen gland, and disappears into the left side of the female gland mass to join the fertilization chamber (Figures 4, 5, 6, 10<sup>(B)</sup>). Before it starts the arch over the albumen gland, it becomes very narrow; the lumen may have a diameter no greater than that needed for the passage of oocytes in single file. The tube is completely lined with columnar cells bearing very strong cilia as tall as the cells. A small swelling, erroneously called the fertilization chamber by MARCUS (1961), is often evident as the duct slips between the common genital duct and the anterior lobe of the albumen gland (Figure 36). An interesting structure, here designated as the post-ampullar gland (Figures 10, 36), starts just forward of this point. This gland begins with a few columnar lining cells which are greatly enlarged to form a narrow glandular strip along the wall of the duct. This glandular strip is composed of 2 types of cells, both completely PAS negative. The most obvious are wide columnar cells with basal nuclei and rounded, spreading glandular heads which narrow abruptly before reaching the lumen. Between these are other cells with slender bases against the basement membrane and enlarged distal regions which contain the nuclei. The distal ends of these cells bear strong cilia and are expanded to form what appears to be a solid ciliated surface on the glandular area. This alternate arrangement of thick glandular cells with basal nuclei and thin cells with expanded ciliated

(E) Editor's note: Figure numbers in *Italics* refer to illustrations on halftone plates, whereas Roman numbers refer to illustrations in the text.

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Figure 4

*Phyllaplysia taylori*

Left aspect of the internal reproductive organs  
Scale line roughly represents 2 mm

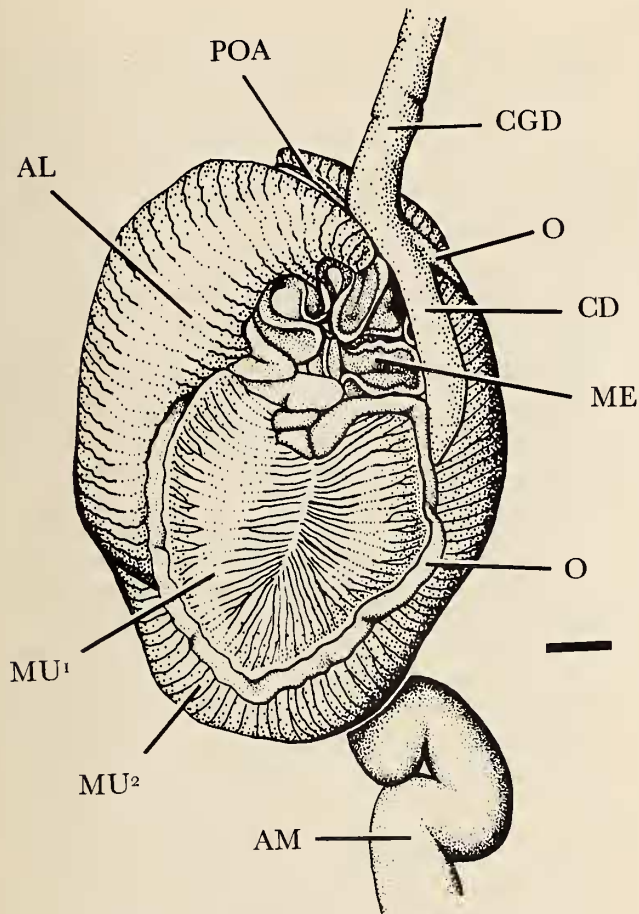


Figure 5

*Phyllaplysia taylora*

Right aspect of the female gland mass. Dorsal edge is to the left in this drawing

Scale roughly represents 1 mm

tips and distal nuclei is characteristic of many of the glandular areas of the gastropod reproductive tract. It was observed by MAZZARELLI (1891) in *Aplysia* and is nicely represented in his figures 35 and 45, Plate III, for the membrane gland.

The width of the post-ampullar gland increases distally until it lines the complete circumference of the post-ampullar duct except for a narrow groove which is reflected against the side of the tube. The gland stains heavily with methyl green, a mucous stain. In stained preparations the color terminates just as the post-ampullar duct enters the fertilization chamber. Cells of similar morphology, but not staining with methyl green, continue through the chamber into the common genital duct.

There is surprising variation in the accounts of ciliation in anaspidean ampullae. MAZZARELLI (1891) notes some cilia in *Aplysia*. EALES (1921) reports that the ampulla of *Aplysia punctata* is unciliated until it constricts to form the post-ampullar duct. MARCUS & MARCUS (1957) state that cilia occur in both of these parts of the coelomic gonoduct in *A. brasiliensis* RANG, 1828, *A. dactylomela* RANG, 1828, and *A. juliana* QUOY & GAIMARD, 1832. THOMPSON & BEBBINGTON (1969) report that  $\frac{1}{3}$  -  $\frac{1}{2}$  of transverse sections of the ampullae of *A. depilans* GMELIN, 1791, *A. fasciata* POIRET, 1789, and *A. punctata* are ciliated. McCauley (1960) and WINKLER (1957) in their major studies of the anatomy of *Phyllaplysia taylora* and *A. californica*, respectively, made no mention of cilia in the coelomic gonoduct. MACFARLAND (1966) indicates, in what are probably the most careful previously published observations on the reproductive system of *P. taylora*, that the ampulla is lined with cilia "throughout the greater part of its extent."

### PALLIAL GONODUCT

Pallial gonoduct is the term used by GHISELIN (1964, 1965) and many others for the reproductive organs intervening between the fertilization chamber and the common genital aperture. It has been assumed that the pallial gonoduct arose originally as a simple epidermal groove or tube which has since evolved complexities and specializations in both structure and function. Although logical, there is as yet little evidence beyond that provided by comparative anatomy to support this assumption.

In anaspideans, the pallial gonoduct is considered to consist of the common genital duct, which in turn gives rise to the female gland mass at its proximal end, the copulatory bursa further distad, and finally the expansion which forms the atrium and atrial gland, before it becomes the external genital groove at the common genital aperture.

#### 1. Female Gland Mass (FGM)

##### a. Topology

No part of the anaspidean reproductive system has given rise to more confusion than the female gland mass. This term refers to the distinct unit formed by the albumen gland, membrane gland, and mucous gland. The complexity of the association of these organs makes it imperative to consider them as a unit before dealing with them individually.

In most anaspideans the female gland mass is an olive-shaped unit stemming from the proximal part of the

pallial gonoduct at its junction with the coelomic gonoduct. Figures 3 and 4 show that in *Phyllaplysia taylori* its position is similar, but the mass is laterally more flattened; the right side bulges convexly against the left wall of the gill cavity, while on the left side the mass is generally concave due to its cramping against the rounded surface of the digestive gland. The common genital duct, the tubular portion of the pallial gonoduct, runs forward from its ventral edge.

The fertilization chamber, shown in Figures 36 and 37, is the structural and functional focal point of the female gland mass. It connects directly to the common genital duct, the 3 glands of the female gland mass, and the seminal receptacle and thus should be considered with them as part of the pallial gonoduct.

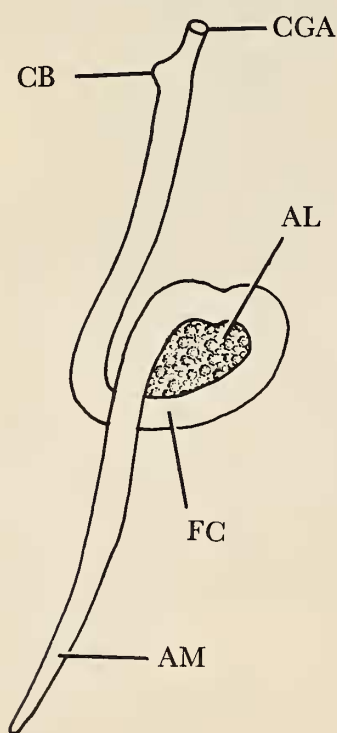


Figure 6

Juvenile condition of the reproductive system in *Aplysia punctata*  
Modified from MAZZARELLI, 1893a. Not to scale

Figure 4 shows the left face of the female gland mass with the seminal receptacle and the anterior part of the ampulla nestled in its concave surface. The ventro-posterior surface is occupied by the translucent white secondary lobe of the mucous gland. The albumen gland, closely matching the mucous gland in texture but of a slightly

yellowish cast, fills the anterior region. The descending loop of the post-ampullar duct marks the albumen gland into ventral and dorsal parts. Dissecting away the ventral part exposes the junction of the descending loop of the post-ampullar duct with the fertilization chamber (Figure 36).

Careful dissection and reconstruction of serial sections have shown that the female gland mass of *Phyllaplysia taylori* is basically 3 sacculations with all their internal surfaces continuous with each other in the area of the fertilization chamber (Figure 36). Each of these 3 pockets develops into a gland whose wall is then folded into increasingly complex patterns. No part of the mass is a true tube separate from the rest of the pallial gonoduct. The "tubes" that have been reported previously are the internal edges of folds.

While the ontogeny of the female gland mass has not been followed, it is possible to visualize how this system may develop. Figure 6 is a modification of MAZZARELLI's (1893) sketch of the hermaphroditic system in a juvenile *Aplysia punctata*. It simply shows a single tube running from the ovotestis to the common genital aperture; the middle of this tube is thrown into a loop within which the albumen gland is found. With some imagination, one can think of one side of the above loop giving off 2 pockets at the fertilization chamber point. One of these pockets would be the membrane gland which then secondarily develops a tortuous folding. The other pocket would be the mucous gland secondarily turning anteriorad in a manner which results in a small right primary lobe and a large left secondary lobe. These processes would result in pockets of the shape suggested by Figures 35 (MU<sup>1</sup> and MU<sup>2</sup>) which shows the routes taken by eggs around the internal edges of these folds of the adult.

The seminal receptacle and albumen gland also open into the fertilization chamber. They are merely arched blind outpocketings. The albumen gland fits in the large dorsal groove between the 2 lobes of the mucous gland (Figure 36).

It is evident that evagination and infolding of simple tube linings, even to a quaternary order, are basic mechanisms by which the organs of the female gland mass and, as shown later, the other reproductive organs in this anaspidean are formed.

#### b. General Discussion

The fertilization chamber is not only the focal point of form and function of the female gland mass; it is also the center of controversy and of conflicting and partly or wholly erroneous reports. There are only 3 original figures of the reproductive system of *Phyllaplysia taylori* in the literature. MACFARLAND (1966) shows only an external

view of the system, McCauley's (1960) figure shows an incorrect arrangement of the organs, but correctly indicates most of the connections to the fertilization chamber. McCauley evidently believed that all of these connections were tubes and that the chamber did not connect directly to its mucous gland. Marcus (1961), despite reference to McCauley's paper, gives a completely erroneous diagram. The slight swelling in the ascending limb of the post-ampullar duct is labeled as the fertilization chamber, whose only exit is into a tubular membrane gland, which in turn connects to a tube running along the female gland mass to the common genital duct. No connections to what should be the albumen and mucous gland are indicated. Marcus & Marcus (1957b) seem to show a similar arrangement in *P. engeli* Marcus, 1955, except here they indicate the seminal receptacle, where "the alien spermatozoa are heaped without any order," attached near the "fertilization chamber" rather than high on the common genital duct. The authors state that the "acidophil albumen and basophil mucous gland communicate with the spermooviduct [emphasis mine] by several connections." Both these latter papers indicate that the only connection between the ampulla and the common genital duct is through a single tube which follows a long course around the female gland mass and has occasional connections with it (Figure 34).

It is not now possible to predict the extent to which the folded arrangement of the female gland mass of *Phyllaplysia taylori* will be found to apply to other anaspideans. The condition may be widespread. The routes in this mass may have formed into true tubes in some species; however, since folds in *P. taylori* have been repeatedly called tubes, the existence of such "tubes" in other anaspideans is highly suspect.

The folded nature of the membrane gland has been reported previously by GUIART (1901) for *Aplysia punctata*, and by MARCUS & MARCUS (1957a) for *A. brasiliensis*, *A. dactylomela*, and *A. juliana*. Interpretation of the membrane gland as a "winding gland" of convoluted tubules seems to be an error that has long persisted. MAZZARELLI's (1891) description, many features of which can be traced to CUVIER (1803), refers to this gland as "twisted like a ball of thread." This view has been maintained by MACFARLAND (1909, 1918), EALES (1921), WINKLER (1957), McCAULEY (1960), and others.

The primary and secondary folding of the mucous gland has not been reported previously. As noted above, all previous authors who have dealt with *Phyllaplysia taylori* have considered this gland to be a tube. MAZZARELLI (1891), GUIART (1901), EALES (1921), and THOMPSON & BEBBINGTON (1969) clearly show it as a tube in *Aplysia punctata*. This is difficult to believe, for (as noted later),

the female gland mass of this species has a special similarity to that of *P. taylori*. MACFARLAND's 1918 work on *Dolabella* mentions the "plication of the walls of this duct," but this refers to the tertiary folding within the gland.

It is now difficult to apply the classic descriptive terms "monaulic," "diaulic," etc. to *Phyllaplysia taylori*'s complex single-tube pallial gonoduct. GHISELIN (1965) has reminded opisthobranch workers that the word "aulic" refers to a tube, and thus words coined from it should refer only to the number of reproductive tubes. Using as a model EALE's (1921) diagram of the *Aplysia punctata* reproductive system, which clearly portrays a tubular female gland mass, GHISELIN has coined the term "oodiaulic" to apply to the reproductive systems of the Anaspidea, the Cephalaspidea of the family Diaphanidae, and the Sacoglossa. GHISELIN (*loc. cit.*) defines the oodiaulic condition as "a variation of the ancestral form in which the nidamental glands have, to a greater or lesser degree, acquired a separate, closed channel by a division of the pallial gonoduct." From a strictly morphological viewpoint, *P. taylori* is monaulic, though perhaps it could be considered as presumptively or functionally diaulic. If GHISELIN's term "oodiaulic" is to be applied, it should be used only with the clear understanding of the slight extent to which the nidamental glands can be considered to have a "separate closed channel."

### c. Phylogenetic Significance

The importance of the arrangement of the organs in the female gland mass has been much neglected. This is partly due to a poor understanding of its anatomy. Its complexity makes it an exceedingly difficult area to dissect, to interpret in serial sections, and to illustrate, while its glandular nature and high mucus content pose special problems to one making histological preparations for study. Most anaspideans have ovoid-shaped female gland masses which have evidently undergone greater compaction than that of *Phyllaplysia taylori*, and which present an external banding which probably reflects further internal complication. The complete internal arrangement of such a female gland mass has never been satisfactorily worked out. MAZZARELLI (1891) shows external views and a sketchy diagram of the internal arrangement for this type of female gland mass in *Aplysia limacina* LINNAEUS, 1758 and *A. depilans* GMELIN, 1791. ANDERSON (1933) and WINKLER (1957) do the same for *A. californica*. MARCUS & MARCUS (1957a) show a similar unit for *A. cervina* DALL & SIMPSON, 1902, *A. brasiliensis*, *A. dactylomela*, and *A. juliana*.

It is here proposed that the type of female gland mass found in *Aplysia californica* be called the "banded-ovoid

type" and the homologous but very different appearing mass in *Phyllaplysia taylori* be called the "flat-pocket" type. It must be emphasized that these are terms of convenience only; the bands of the banded-ovoid type may indeed represent parts of extended pockets, but this is not externally apparent and has not been well confirmed internally.

It is interesting to note the occurrence of the different female gland mass types in the anaspideans. As would be expected, other *Phyllaplysia* species seem to have the flat-pocket type. The very sketchy figures of MARCUS & MARCUS (1957b) and MAZZARELLI (1893b), for *P. engeli* and *P. lafonti* (FISCHER, 1870) respectively, indicate this. PRUVOT-FOL's (1954, 1960) exceedingly poor diagram of *Petalifera petalifera* (RANG, 1828) suggests a similarity to *Phyllaplysia*. GUIART's (1901) diagram of *Notarchus punctatus* PHILIPPI, 1836 and MACFARLAND's (1918) diagram of *Dolabella agassizii* MACFARLAND, 1918 also suggest the flat-pocket type. The female gland mass of *Dolabrifera* has not been adequately figured; PRUVOT-FOL's (1960) diagram of the reproductive system of an unspecified *Dolabrifera* is meaningless.

Most interesting comparisons can be made between *Phyllaplysia taylori*, *Aplysia punctata*, and *Akera bullata* MÜLLER, 1776. MAZZARELLI's (1891) and EALES' (1921) figures for *Aplysia punctata* show external and internal features which very strongly suggest that the female gland mass is of the flat-pocket type, and is perhaps even simpler than that of *P. taylori*. INGIER's (1906) work on *Akera bullata* lacks diagrammatic or functional layouts of the reproductive system, but her excellent drawings of gross anatomy and serial sections show features amazingly similar to those of *P. taylori*. The albumen gland even fits between the lobes of the mucous gland in the same manner. *Akera*, although very probably divergent in several ways from the ancestral anaspideans, is generally accepted as an anaspidean with many primitive features (GUIART, 1901; BOETTGER, 1954; BEEMAN, 1968a; etc.). This suggests that the flat-pocket type of female gland mass is more primitive than the banded-ovoid type. As far as presently is known, all species of *Aplysia* other than *A. punctata* have the banded-ovoid type. It would be interesting to know if *A. parvula* GUILDING in MÖRCH, 1863, the only other member of the subgenus *Pruvotaplysia* to which *A. punctata* belongs, has the flat-pocket type of female gland mass. EALES (1960) did not mention any reproductive features in establishing this subgenus, but she considers all of the characters listed as features of the subgenus to be primitive ones. If both *P. taylori* and *A. punctata* are presumptively oodialeuc, they are closer to each other in reproductive structure than either species is to other *Aplysia* species.

The features of the individual organs of the female gland mass can now be considered separately. It should be noted that many authors, such as PRUVOT-FOL (1960) and MACFARLAND (1966), do not recognize a distinction between membrane gland and mucous gland.

## 2. Albumen Gland (AL)

The "albumen" gland of *Phyllaplysia taylori* is a large, yellowish-white caecum extending from the fertilization chamber. Its anterior end forms most of the forward part of the female gland mass; its posterior section fits between the dorsal edges of the 2 mucous gland lobes. Most of its external features are discussed in the preceding section and illustrated in Figures 4, 5, 26, and 36. Its position and connection have been noted correctly by almost every author except MARCUS (1961).

The large, irregularly-shaped lumen often has a large secondary fold running along its dorsal and anterior edge (Figure 36). The walls are composed of thin, longitudinal, tertiary folds which extend into the lumen as sharp-edged ridges. These folds are composed of broadly columnar, strongly PAS-positive, unciliated cells. The elliptical basal nuclei are about  $\frac{1}{4}$  the height of the cells; each contains a single distinct nucleolus. The upper  $\frac{2}{3}$  of each cell are densely filled with secretion granules. A dense connective tissue fills the thin spaces between the folds and the squamous epithelium which forms a simple, tight sheath over the entire organ.

The gland narrows sharply as it enters the fertilization chamber. As noted by MACFARLAND (1966), this neck is lined with a low ciliated endothelium. The cilia, averaging  $11\mu$ , are up to twice the height of the cells which bear them. MACFARLAND also reports large nerve cells "projecting into this epithelium at intervals."

## 3. Membrane Gland (ME)

This small gland forms the right-anterior section of the female gland mass (Figures 5, 26, 36). Its spatial arrangement and associations with the remainder of the gland mass have been discussed above. The tangled external appearance caused by its secondary folding has led many authors to call it the "winding gland." Others have referred to it as a special part of, or have failed to distinguish it from, the mucous gland. It has many structural and histological resemblances to the mucous gland and may only be a specialized part of that organ. Like the mucous gland it is moderately PAS positive. The membrane gland differs from the mucous gland primarily in its position and in its highly convoluted folding. Its entrance is formed

by a fold which gives the impression of a tube leaving the fertilization chamber (Figure 36).

Methyl green staining reveals vividly that the membrane gland is not uniformly glandular. The proximal, anterior region stains darkly while the distal, posterior folds do not stain (Figure 5). A tortuous line of unstained tissue indicates the edge of the oviducal groove. This edge is composed of cuboidal cells with very powerful cilia 2 to 3 times the height of the cells.

The endothelium lining the glandular anterior region of the membrane gland has the same arrangement of glandular and ciliated cells described for the post-ampullar gland. The cilia are up to twice the height of the cells which bear them. Ciliation is less dense away from the groove. Toward the non-staining posterior region of the membrane gland, the ciliated and glandular cells become sparser. The oviducal groove continues from this area into the primary lobe of the mucous gland.

#### 4. Mucous Gland (MU)

The mucous gland is a semi-translucent white organ which forms the greater part of the female gland mass (Figures 4, 5, 36). Due to its complex folding, discussed in the preceding section, it forms 2 unequal, connected lobes, the smaller being visible on the left side. Since the smaller lobe is the first in the sequence out of the membrane gland, it is here designated as the primary mucous gland lobe (MU<sup>1</sup>); the larger lobe is thus the secondary lobe (MU<sup>2</sup>).

A strongly ciliated groove follows the inside edges of the gland (Figure 13). This is an extension of the oviducal groove from the membrane gland. After following the double U-shaped route indicated by Figures 35 and 36, the oviduct leaves the gland and becomes part of the spermoviduct of the common genital duct. The oviducal groove in the mucous gland is composed of a simple ciliated columnar endothelium. A group of special gland cells, resembling those of the post-ampullar gland but strongly PAS-positive, are present along one of the inner sides of the open groove.

The oviducal groove opens along its entire course on one side to the non-ciliated, glandular region which comprises the bulk of the mucous gland. The glandular region is composed of third and fourth order folds which form ridges running diagonally to the oviducal groove. The cells are only moderately PAS-positive. When the cells are not secreting, the general histology resembles that of the membrane gland. Cell structure and staining properties differ from those of the albumen gland. Methyl green stains the mucous gland green or blue-green; it leaves the albumen gland white in alcoholic solutions. Two

cell types are found in the walls of the mucous gland. The larger cells are columnar with occasional vacuoles near the base and large spherical nuclei about halfway up the cell body. The smaller cells possess nuclei about  $\frac{1}{2}$  the diameter of those in the larger cells; the cells are broad distally and apparently have slender basal extensions. During the secretory phase the vacuoles expand to almost fill the large cells and swell the cells several fold.

Connective tissue bearing blood sinuses lined with squamous cells fills the small spaces under the folded epithelium lining the mucous gland. The entire organ is encased in the usual sheath of very thin squamous epithelium.

#### 5. Seminal Receptacle (SR)

The seminal receptacle is a blind sac. While it opens into the fertilization chamber in *Phyllaplysia taylori*, this is not the case in all anaspideans. My dissections of *Aplysia californica* show that its sole opening is at a point over  $\frac{1}{2}$ -way up the common genital duct in that animal. Positional variations of this type are the basis for a long-standing dispute in the literature. A connection between the seminal receptacle and the fertilization chamber in aplysids was first reported by CUVIER (1803) and this duct later came to be known as the "duct of Cuvier." This term was then used in reference to aplysids in which the seminal receptacle opened at a point very near the fertilization chamber and thus "duct of Cuvier" was virtually synonymous with the term "seminal receptacle duct." MAZZARELLI (1891) figured the seminal receptacle duct of a "generalized" aplysid as opening into the proximal region of the common genital duct. He used the term "duct of Cuvier" to refer, not to the seminal receptacle duct, but to a hypothetical connection between the seminal receptacle duct and the fertilization chamber. However, MACFARLAND (1909) continued to consider "duct of Cuvier" as synonymous with seminal receptacle duct and he applied the former term even to a seminal receptacle duct which opened into the common genital duct far from the fertilization chamber. The resulting confusion led MARCUS & MARCUS (1957) to declare that there was a "serious discrepancy between MACFARLAND's and our observations" concerning *Aplysia cervina*. Actually the figures of MARCUS & MARCUS (*op. cit.*) and MACFARLAND (*op. cit.*) agree perfectly on this point. The term "duct of Cuvier" has only caused continued confusion and it should be abandoned by malacologists.

The point at which the duct of the seminal receptacle opens into the pallial gonoduct varies in different anaspideans. This also raises a question as to whether the organs

referred to as seminal receptacles are really homologous structures throughout the anaspideans and other opisthobranchs.

The internal structure of the seminal receptacle is shown in Figures 27, 28, 29, 30 and 31. A very thin outer sheath covers a thick underlying muscular layer. This, in turn, is lined with a single unciliated columnar endothelium. Variations in the height of the endothelium form longitudinal ridges and furrows adjacent to the lumen. The nuclei lie basally in the endothelial cells and are about  $\frac{1}{2}$  the height of the shorter cells. Scanning electron microscopy reveals that inner tips of these endothelial cells are rounded, equipped with microvilli, and secrete material into the lumen.

If the gastropod seminal receptacle is defined as the female region containing oriented sperm (*e. g.*, GHISELIN, 1965), this structure fits the definition well. Figure 29 shows sperm packed in parallel rows in the furrows of the receptacle. The tips of these sperm are embedded into the endothelial cells. The development of the muscle coat and the endothelial ridges diminishes as the receptacle narrows to form its arched duct.

## 6. Common Genital Duct (CGD)

The common genital duct (= large or wide hermaphroditic duct) is a tubular continuation of the pallial gonoduct from the fertilization chamber and the pallial oviduct to the common genital aperture (Figures 4, 5, and 36). Externally it appears as a single duct in *Phyllaplysia taylori*, but it is actually composed of 2 distinct and parallel ducts, the pallial spermoviduct and the copulatory duct (= vaginal channel), plus an intervening space. These 2 ducts are incompletely separated by 2 infolded ridges of the lining endothelium, here called the spermoviduct fold and the copulatory fold (Figures 15 and 36). THOMPSON & BEBBINGTON (1969) also found this duct to be incompletely divided in *Aplysia punctata*, *A. fasciata*, and *A. depilans*. This arrangement evidently does not occur in all anaspideans. In some species of *Aplysia* much of the proximal part of the common genital duct is reported to be completely divided (*cf.* MARCUS & MARCUS, 1957a, fig. 16). Functionally and phylogenetically, the divided pattern is probably a more advanced condition.

The spermoviduct fold is the largest of the internal folds and vertically divides the common genital duct throughout its proximal part. Its free edge is either broad or recurved toward the copulatory duct. At the level of the copulatory bursa duct (Figure 4), the spermoviduct fold is pressed against the inner wall of the common gen-

ital duct to form an internal seminal groove which makes 2 counter-clockwise turns and then reverses for  $\frac{1}{2}$  of a clockwise turn as the common genital duct coils tightly between the copulatory bursa duct and the atrium. These turns are partly visible on the outside of the system (Figure 4). The internal seminal groove then avoids the lumen of the atrium and makes 2 clockwise turns before it reaches the exterior at the common genital aperture.

A third fold, here called the egg-string guide, is prominent at the proximal end of the pallial gonoduct, but tapers out and disappears before the level of the entrance of the copulatory bursa duct. This blunt fold is found in the wall of the spermoviduct. A true continuation of the pallial oviduct from the female gland mass (Figures 13 and 26) can be followed within the spermoviduct (Figure 15) until this small fold fades out.

The copulatory bursa duct enters the pallial gonoduct where the latter becomes tightly coiled. Internally the copulatory bursa duct enters the copulatory duct (Figure 23), but it is at this point that the above-mentioned seminal groove begins and part of the space of this spermoviduct merges into the copulatory duct.

The copulatory duct is more distinct in its entire route. Proximally it forms a caecum slightly below the fertilization chamber (Figure 26) and then extends as a duct distad, forming the atrium by great expansion of one side; and finally it opens to the exterior by a short duct surrounded by the final coils of the internal seminal groove. This opening of the copulatory duct and the modified spermoviduct to the outside of the body is the common genital aperture. The seminal groove continues anteriorad from this aperture as the external genital groove.

The histology of the ducts helps to define their extent, especially in cross-section. The same special endothelium of broad, columnar secretory cells and slender, distally expanded ciliated cells described for the post-ampullar gland and the membrane gland also lines the spermoviduct. This tissue matches what GHISELIN (1965) has referred to as the "prostate." It will be referred to here as the spermoviduct gland. In typical cross-section views (Figure 15), this special endothelium starts near the oviduct side of the spermoviduct fold and extends to the base of the copulatory fold. Its ciliary and secretory nature is most strongly developed in the oviduct region of the spermoviduct (Figure 14). In one specimen, the glandular cells were 100 $\mu$  long and 10 $\mu$  wide, while the ciliated cells, also 100 $\mu$  long, were only 1 or 2 $\mu$  wide for most of their length. Such cells have been well illustrated by MAZZARELLI (1891) and LLOYD (1952). The cells

become lower and more cuboidal toward the base of the copulatory fold.

The spermoviduct gland represents a continuation of the oviductal gland tissue; the cells are similar histologically, and are also strongly PAS positive. The spermoviduct gland becomes restricted distad to the internal seminal groove, and finally disappears about the level of the atrium. The remainder of the common genital duct, mostly copulatory duct, is lined with columnar-cuboidal ciliated cells. A few greatly expanded, highly basophilic cells are found in the region of the egg-string guide.

The entire common genital duct is encased in a circular layer of smooth muscle. The thickness of this muscular coat diminishes distally except for special development near the atrium and common genital aperture. A simple squamous epithelium covers the outside of the tube.

### 7. Copulatory Bursa (CB)

The copulatory bursa is an almost perfectly spherical organ connected to the upper part of the common genital duct by a thin muscular tube (Figures 4 and 24). Tissue layers present include the usual thin outer epithelium, a middle layer of connective tissue and muscle, and a lining of columnar endothelium (Figure 25). This sphere is capable of great expansion but, unlike the atrium, it possesses no special folds to accommodate this.

The endothelial cells lining the organ are very distinctive. The entire cell shows a strong pattern of longitudinal fibers; these are especially well developed in the upper and lower quarters of the cell body which seem to be devoid of all other structures and which stain a pale mixture of red and blue in hematoxylin and eosin preparations. The fibers are especially dense proximally, and form a distinctive basal band in which visible cell boundaries are lacking. This band resembles the basal fibrillar apparatus which WILSON (1925) referred to as tonofibrillae and described as having a skeletal support function within tissues. The center  $\frac{1}{2}$  of these cells is less dense and takes a much more definite basophilic stain. A very distinctive elongate nucleus occurs in the lower region of this section. A clear vacuole, which appears to be part of the nucleus, is apparent at its basal end. This vacuole is enlarged beyond the width of the nucleus in a few cells, is about nuclear width in the majority of cells, and is apparently discharged in others. From 1 to 4 clear vacuoles are usually evident in the upper part of the basophilic region. Some of these vacuoles coalesce; a very few seem to be discharging through the dense upper quarter of the cell to the lumen of the bursa.

The bursa is rarely empty. Usually it contains a strange, layered mixture consisting of granules, spermatozoa in various stages of breakdown, amorphous material, and

yellow-red to dark-red oil droplets (Figure 23). The amorphous material frequently forms large, rounded, densely packed nodules.

The copulatory bursa duct is a muscular tube lined with a simple cuboidal endothelium bearing cilia 2 or 3 times the cell height. The nature of its connection to the common genital duct has been discussed in the section on that duct.

### 8. Atrium (AT)

The atrium or genital atrium is a large expansion of the posterior side of the copulatory duct near the common genital aperture (Figure 4). It is a loosely walled sac with a posterior tip, often curved ventrally.

The outer wall is a muscular layer with a thin squamous epithelial covering. The inner surface is an enormously convoluted columnar endothelium (Figure 11). A "cuticle" covers the lumen surface of the cells. This layer is about  $\frac{1}{4}$  to  $\frac{1}{3}$  the height of the cells, with occasional areas which are very much thicker or thinner. Due to the presence of this layer it is not possible to discern if this surface shares the ciliary cover found in almost all of the rest of the common genital duct. The luminal endothelial cells are entirely basophilic except for a slightly eosinophilic basement membrane. The lumen ends of the cells are expanded. The nuclei are central and elongate. A clear vacuole-like structure is occasionally seen next to the basal end of the nucleus.

The duct from the atrial gland enters the left side of the atrium. The internal genital groove bypasses the atrium at the anterior end of the lumen.

Two muscular bands close the 2 main atrial openings. A very heavy muscular semi-circular band, here called the atrium basal muscle (ABM), is found on the copulatory duct at its proximal, anterior entrance. A sphincter encircles the short portion of the common genital duct which forms the distal, dorsal exit of the atrium.

The development of the atrium is highly variable among different anaspideans. A small swelling where the copulatory bursa duct joins the common genital duct is designated as the "lateral pocket, or bursa seminalis" by EALES (1921) and referred to as the "clustered gland" by MARCUS & MARCUS (1957a). THOMPSON & BEBBINGTON (1969) refer to a similar swelling in *Aplysia fasciata* and *A. punctata* and an extended glandular area in *A. depilans* as a prostate gland. These structures are probably homologous with the atrium of *Phyllaplysia taylori*, as their walls bear a glandular tissue which seems to be identical with that of the atrial gland in my material.

A similar small swelling is shown as the "réservoir séminal" for *Akera bullata* by GUIART (1901). This is evidently the same structure that INGIER (1906) labels

the "Prostatadrüse." The large organ which MACFARLAND (1918) calls the "seminal receptacle" in *Dolabella agassizi* matches the description and illustration of the atrium in *Phyllaplysia taylori*. It is obvious that knowledge of this organ leaves much to be desired.

### 9. Atrial Gland (ATG)

The atrial gland in *Phyllaplysia taylori* is a distinct ovoid organ attached to the left side of the anterior, proximal end of the atrium (Figure 4). Its single duct connects directly with the atrial lumen near the atrium basal muscle. In living animals, the yellowish color of the gland contrasts with the greenish-white of the atrial walls.

The atrial gland can be recognized instantly in sections by its compact clusters of acini entirely lined with basophilic glandular cells (Figure 12). There are typically 3 rows of these acini encircling and opening into a central lumen. A thin layer of connective tissue surrounds the acini and a simple squamous outer epithelium covers the outside of the organ.

The acini are lined with a simple endothelium of large columnar cells with spherical basal nuclei each containing 1 or 2 distinct nucleoli. The upper halves of these cells are expanded and contain numerous clear, coalescing secretory vacuoles. Cilia, as long as the cells, line all of the internal ducts and part of the main duct to the atrium. The cells of this main duct are distinctly different from those of the gland; they are very much smaller, non-secretory, and grade from columnar to cuboidal in shape.

The atrial gland is often confused with the atrium itself by authors dealing with other anaspidean species.

### EXTERNAL GENITAL GROOVE (GG)

The external genital groove is a continuation of the pallial spermoviduct, and extends along the outer body wall

from the common genital aperture to the tip of the penis. The groove is not homogeneous throughout, but possesses 2 distinct regions. The first runs along the right dorsal surface of the animal from the common genital aperture to the opening of the penial sheath near the right rhinophore (Figure 3). A flap extending from the dorsal left edge of the open groove forms this region into a functional duct. Most of the circumference of the duct is lined with a simple, strongly ciliated epithelium of cuboidal to columnar cells. The nucleus is central in each cell. Occasional glandular cells similar to those in the PAS-positive mucous glands of the skin occur. The second section of the external genital groove runs into the penial sheath, along its wall, and then along the glans penis (Figures 19, 20, and 21). Cross-sections of the glans penis show that this section of the groove forms a duct by inrolling of the groove and an expansion of its bottom. The groove is lined with very small cuboidal cells; those along the bottom of the groove bear cilia 2 or 3 times their height.

### PENIS (P)

The penis of *Phyllaplysia taylori* (Figure 19) is composed of the penial sheath (termed preputium by some authors) and the glans penis (termed penis by most authors). The sheath is obviously a tubular inpocketing of the outer body wall surface; it even has fine parallel stripes for about  $\frac{1}{3}$  of its extent which match the pigment of the dorsal stripes. When inrolled, the sheath projects into the hemocoel along the brain; it opens to the surface by the penial sheath aperture under the right eye. The glans penis rests coiled within the sheath, attached to the latter at its inner end. The glans is a flattened organ with the genital groove running along one edge. Powerfully muscular, the glans is composed mainly of crossbedded erectile tissue. Both the sheath and the glans are armed with cuticular-tipped spines. These spines are considered to have taxonomic

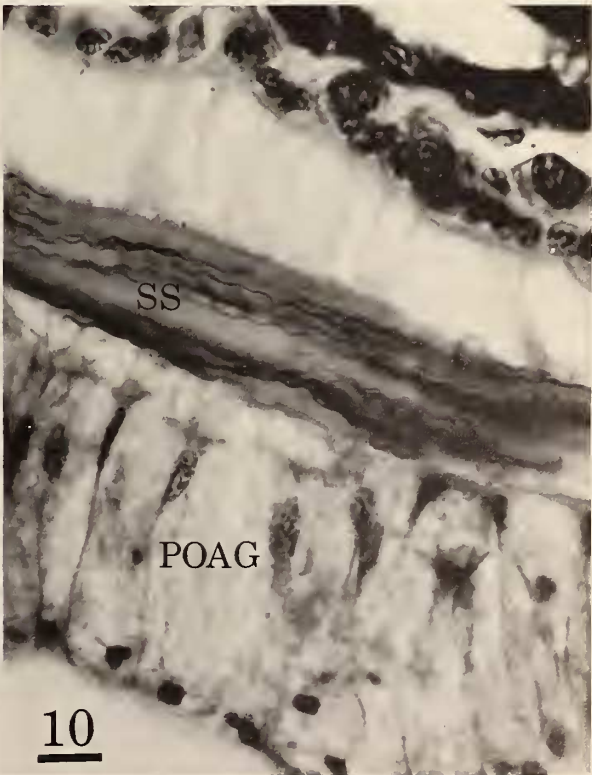
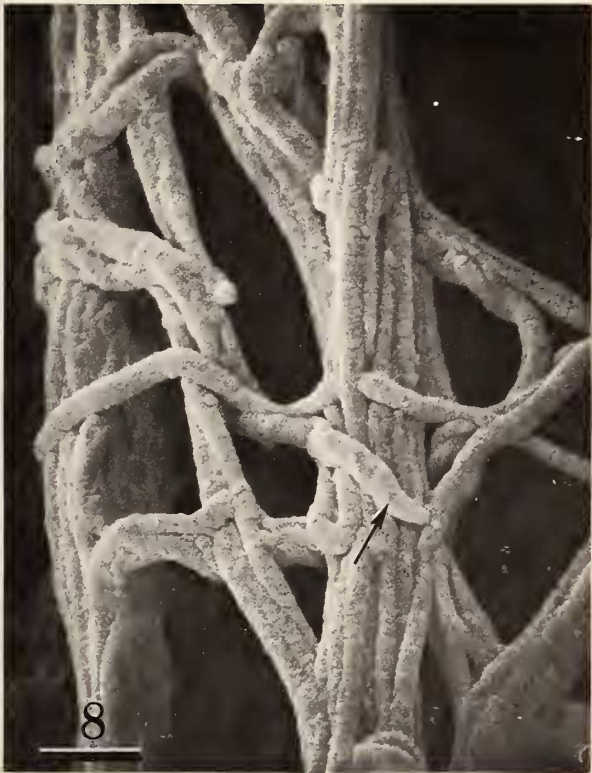
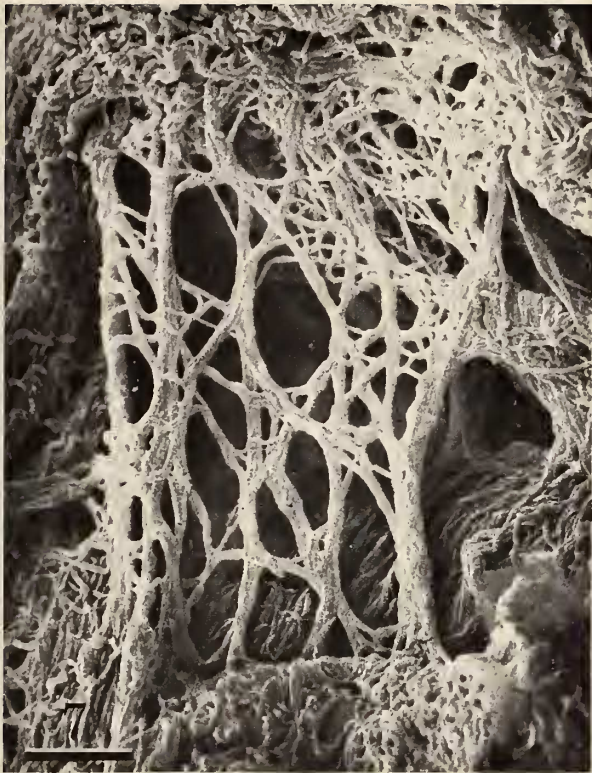
### Plate Explanation

Figure 7: Scanning electron micrograph of ampullar contents in *Phyllaplysia taylori*. The seminal mass has been gently teased apart. 20° beam angle Scale line represents 10μ

Figure 8: Same area as Figure 7 but at higher magnification. Note the parallel bundles of sperm filaments. Arrow indicates the anterior tip of one spermatozoon Scale line represents 2μ

Figure 9: Sperm bundles and one oocyte in the post-ampullar duct. Mayer's haemalum and triosin. Scale line represents 25μ

Figure 10: A seminal strand fixed while moving in the post-ampullar duct along the ciliated surface of the post-ampullar gland. Haematoxylin and eosin Scale line represents 8μ





importance. A powerful penis retractor muscle, originating on the hemocoel floor near the genital atrium, is inserted into the base of the glans penis (Figures 3 and 20). In contrast to many other opisthobranchs, no prostate is associated with the penis. If a prostatic function exists it may be carried out by the post-ampullar gland or the spermoviduct gland.

GHISELIN (1964) has pointed out that the position of the penis on the side of the head is probably a primitive condition. He feels that the original position of the opening of the pallial gonoduct was inside the mantle cavity, and that the forward position of the penis is an evolutionary response to conditions where the gonoduct opening was blocked by the shell. The retention of the penis in this position in the anaspideans is not surprising, as members of the group show numerous primitive features; some even retain a well-developed shell (*e. g.*, *Akera*). The possession by the sacoglossans of a penis lying in a similar position but equipped with a closed ejaculatory duct may represent a higher development of this pattern; it supports the idea that all or part of the Sacoglossa arose from an ancestral stock near the anaspidean line.

## II. FUNCTIONAL MORPHOLOGY OF THE REPRODUCTIVE SYSTEM

The reproductive system of an anaspidean such as *Phyllaplysia taylori* may contain, simultaneously, the following: female gametes at various stages of development ranging from oögonia to fertilized eggs borne in the egg string; endogenous male gametes ranging from spermatogonia to morphologically mature sperm; and exogenous sperm received as a result of copulation. Some of these may be present simultaneously in such areas as the fertilization chamber and common genital duct. For even an elementary understanding of reproductive function in the animal one must know: the morphology of the reproductive tract; the paths of movement of the 3 categories of gametes present (which requires the ability to distinguish exogenous from endogenous sperm); and the events which occur in each part of the reproductive tract. The morphology is covered in the first section of this paper; function is considered below.

## METHODS AND MATERIALS

Animals used in functional morphology studies were freshly obtained from Elkhorn Slough. After the start of experimentation they were kept in the large outdoor seawater tanks, previously described, at Hopkins Marine Station. Living animals and fresh or fixed and sectioned

tissues were used. Most of the relaxation, microdissection, fixation, and microtechnique methods were described in the anatomy section.

Tritiated thymidine autoradiography was a very useful method for distinguishing exogenous from endogenous sperm and for determining the translocations of labeled sperm. MONESI (1962), LIMA-DE-FARIA & BORUM (1962), and others have found that the most mature germ cells that can incorporate this label are the primary spermatocytes and primary oocytes in premeiotic DNA synthesis. Thus spermatozoa bearing this label must have acquired it at their point of origin, the ovotestis. The subsequent movement of such labeled sperm can be detected by autoradiography. Details of my autoradiographic experiments to determine the exchange and storage of sperm within *Phyllaplysia taylori*, and an outline of some highlights of the following results, have been previously reported (BEEMAN, 1970c).

## MOVEMENT AND STORAGE OF ENDOGENOUS SPERM

There is a steady movement of spermatozoa from the lumen of the ovotestis lobes to the ampulla. While the exact mechanism of this movement is not clear, several methods can be postulated. 1) The sperm may move out by their own actions. If this occurs, it must be by groups of sperm rather than by individual ones, for the size and organization of sperm clumps in the ampulla indicate that sperm temporarily remain in the parallel groups in which they developed in the ovotestis. 2) The sperm could be moved out by the pressure created by the increasing volume of sexual products in the ovotestis. 3) The sperm may be moved out by contraction of the ovotestis wall or body wall. There is no evidence to indicate that the ovotestis is capable of any active contraction.

After the sperm is gathered by the tiny tubules of the pre-ampullar duct (Figure 4), it is stored in the ampulla. The increasing volume of sperm greatly distends the ampulla. The pearly white ampulla filled with sperm is visible through the ventral body wall in most animals at most times. While the sperm in a given bundle show a parallel orientation, the bundles themselves stored in the ampulla are oriented at random (Figures 7, 8, 9, and 36). Unlabeled sperm in the ampulla are replaced by labeled sperm within 30 days after injection of tritiated thymidine. Allowing for spermiogenesis time (*ca.* 10 days, BEEMAN, 1970a), this would indicate that sperm, in animals allowed to copulate, remained in the ampulla less than 20 days (BEEMAN, 1970c). Various impurities such as

yolk particles, single oocytes, abnormal sperm, and ameobocytes are occasionally seen mixed with the sperm (Figure 9).

WINKLER (1957), who diagrams and discusses the copulatory bursa as a seminal vesicle (*cf.* my Figure 33) in *Aplysia californica*, nevertheless shows the ampulla distended with stored sperm in his photograph of the gross anatomy of the reproductive system. WINKLER's diagram, at this point only, follows that of EALES (1921) for *A. punctata* (Figure 32). GUIART (1901) felt that the seminal receptacle in *A. punctata* was a seminal vesicle. However, most authors dealing with anaspideans indicate that the ampulla is the location of endogenous sperm storage. Even on the basis of the present radiolabeling alone, I am convinced that the ampulla is the only true seminal vesicle in *Phyllaplysia taylori*, and the same seems highly likely in all other anaspideans as well.

Peristaltic waves of contraction often seen in dissections of *Phyllaplysia taylori* force the ampullar sperm into the post-ampullar duct. As the spermatozoa are forced further into the narrowing post-ampullar duct ciliary action is added to the muscular action and the sperm are aligned into a distinct seminal thread or strand in which sperm bundles now show parallel orientation (Figure 10). The post-ampullar gland cells evidently secrete mucoid material which serves to fill the interstices of, and bind together, this thread of parallel packed sperm; it may also serve to lubricate its passage. Thus the post-ampullar gland very likely has a prostatic function.

The seminal strand (Figures 10, 16, 26, and 36) is a very distinct structure by the time it reaches the fertilization chamber. This cohesiveness of the thread allows the endogenous sperm to be moved by strong ciliary action across the edge of the fertilization chamber to the axial edge of the spermooviduct fold with little chance of mixing with other sexual products (Figure 36). Folds in the wall of the fertilization chamber also seem to have a valve-like action in helping to guide the various sexual products (Figure 26). Vivisections, and the contrast of labeled exogenous and unlabeled endogenous sperm *in situ* in sections of fixed animals, showed clearly that the relatively tough seminal strand is not an artifact caused by

the contraction of an encasing tube, and that it is a purely endogenous structure. Having established this by autoradiography and vivisection it became possible to use the easily recognized features of the endogenous seminal thread as a morphological label to supplement or replace the radio-labeling. This was one of the most valuable results of the study for it provided a means of confirming some of the results obtained with radiolabeled animals through using unlabeled individuals, and permitted the gathering of information not available from the radiolabeled series.

I have not seen oocytes or clumps of yolk particles, both of which are mixed with the sperm in the ampulla, in the seminal thread or exogenous sperm. They are evidently excluded as the thread is formed, and comprise part of the debris which ciliary action sweeps through the fertilization chamber into the female gland mass duct; here it forms some of the material in the "pseudo-eggstrings" that some vivisected animals are seen to produce. It is not clear where these pseudo-eggstrings are eliminated. I have seen this material carried up to the spermooviduct by ciliary action and loosely piled in the outer regions of the common genital duct. It seems likely that this material is normally taken into the copulatory bursa, agglutinated, and partially destroyed. The formation of pseudo-eggstrings suggests that the separation of male and female functions in the fertilization chamber is at least partially mechanical without associated chemical or nervous mechanisms.

MAZZARELLI (1891) did not find a direct connection between the fertilization chamber and the common genital duct; however, he represented such a connection by the dotted line in his generalized diagram of the *Aplysia* reproductive system. His belief that such a duct existed and served only for exogenous sperm led him to deduce and to state clearly that endogenous sperm follow the same route as the eggs through "l'oviduttodeferente," by which he meant both the oviduct groove running through the female gland mass and the spermooviduct running up the common genital duct. This point was corrected by EALES (1921), who evidently based her conclusions on anatomical deduction, but her diagram (*cf.* my Figure

### Plate Explanation

Figure 11: A section of the genital atrium of *Phyllaplysia taylori*. Mayer's haemalum and eosin.

Figure 12: A section of the atrial gland of *Phyllaplysia taylori*. Mayer's haemalum and eosin.

Figure 13: A section of the mucous gland of *Phyllaplysia taylori*, showing the folds of secretory tissue and the oviduct. Haematoxylin and triosin.

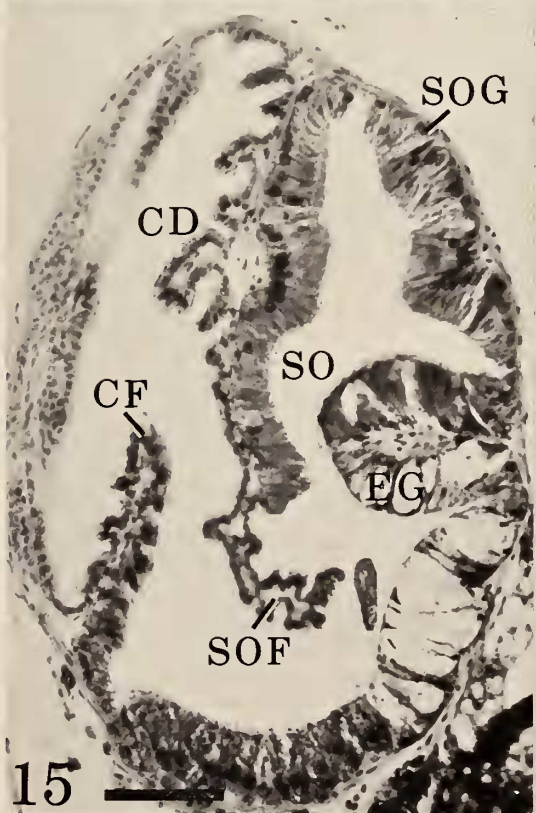
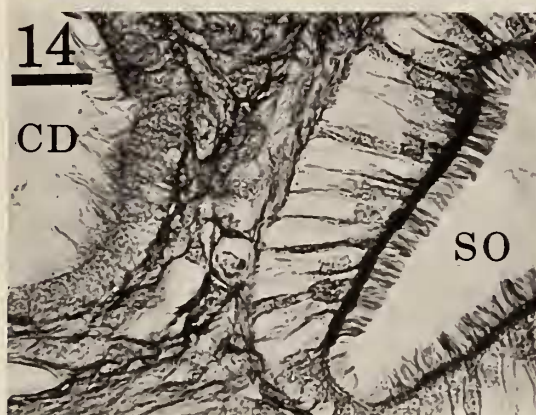
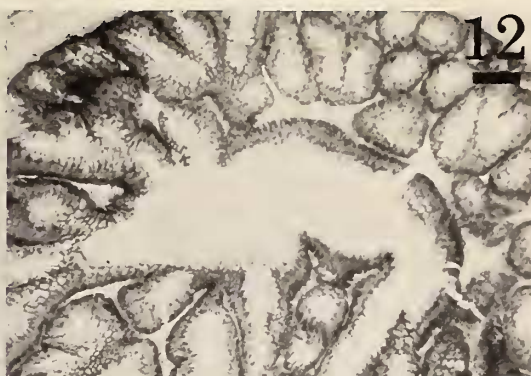
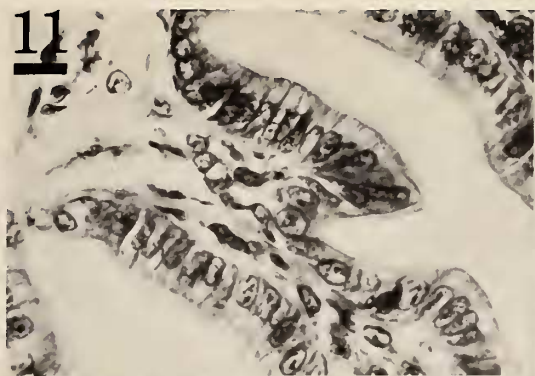
Figure 14: A section from the common genital duct of *Phyllaplysia taylori*, showing the strong cilia of the copulatory duct. Mallory's triple stain.

Figure 15: A cross-section of the common genital duct of *Phyllaplysia taylori*. Haemalum and triosin.

Figure 16: Similar to Figure 15, but showing the ciliary contact between the tip of the spermooviduct fold and the seminal strand.

Figure 17: A cross-section of the common genital duct of *Phyllaplysia taylori* killed while the egg-string was moving up the spermooviduct. Mayer's haemalum.

Scale lines: 10 $\mu$  in Figures 11, 14, 16; 100 $\mu$  in Figures 12, 13, 15, 17.





32) shows the endogenous sperm moving from the fertilization chamber to the copulatory duct instead of to the spermoviduct. She may have been misled by some of MAZZARELLI's drawings, which are confusing since he was not able to distinguish clearly between exogenous and endogenous sperm.

The seminal strand typically moves up the spermoviduct attached to the tip of the spermoviduct fold (Figures 15, 16, and 36). In action, this tip is held against the spermoviduct wall near the outer edge of the egg guide fold; the concave tip and the curving surface of the wall form a tube which encloses the seminal thread. Only ciliary action could be involved in the propulsion of the thread at this point. Examination of Figure 15 shows that muscular action could only be applied laterally and this would have the effect of bending the spermoviduct fold rather than applying forward motion to the thread. The common genital duct can be seen through the body wall in the intact animal. Although fully capable of powerful muscular action, the common genital duct did not show any waves or upward contraction when I watched it in several different animals during copulation.

The spermoviduct gland appears to contain a variety of different gland cells. Only the oviducal region of this gland has a strongly positive PAS reaction; the region where the seminal thread contacts the glandular wall is PAS negative. Some of the cells appear clear in hematoxylin and eosin preparations (Figure 15); possibly these cells provide lubrication for the seminal thread.

A distinct change occurs at the level of the copulatory bursa duct. The spermoviduct gland tapers to an end at about this point and the tip of the spermoviduct fold becomes less distinct. A lateral outgrowth, which encloses the penis tip of the mate, develops on the spermoviduct fold. This deepens the apparent position of the seminal thread in the spermoviduct (Figure 22). At about this point the spermoviduct becomes the internal seminal groove and the spermoviduct gland fades out. The seminal thread moves deep into this groove and follows its tortuous route to the common genital aperture.

It must be stressed that in being moved from the fertilization chamber to the common genital aperture, the outgoing seminal thread has normally completely bypassed the seminal receptacle, the copulatory bursa, the copulatory duct, the atrium, and the atrial gland (Figures 36 and 37). Emerging from the common genital aperture, the seminal thread merely continues forward along the external body wall, carried in the bottom of the external genital groove (Figure 21). Ciliary action propels the thread directly to the tip of the penis which is extended in copulation.

## COPULATION

Copulation normally occurs between 2 animals facing in opposite directions on the surface of *Zostera* leaves. The animals crawl toward each other and each bends to the right so that their right anterior quadrants overlap. The penis of each is now projected and inserted into the common genital aperture of the mate. The usual arrangement is the reciprocal exchange of sperm between the 2 individuals, but one-way transfers and mating triangles are occasionally seen.

Copulation in *Phyllaplysia taylori* was observed to occur in the field throughout the year. Individual *Phyllaplysia* were observed to copulate repeatedly over a period of months in study tanks. The smallest copulating animal observed was 13 mm (66 mg) and this is probably not much above the minimum size.

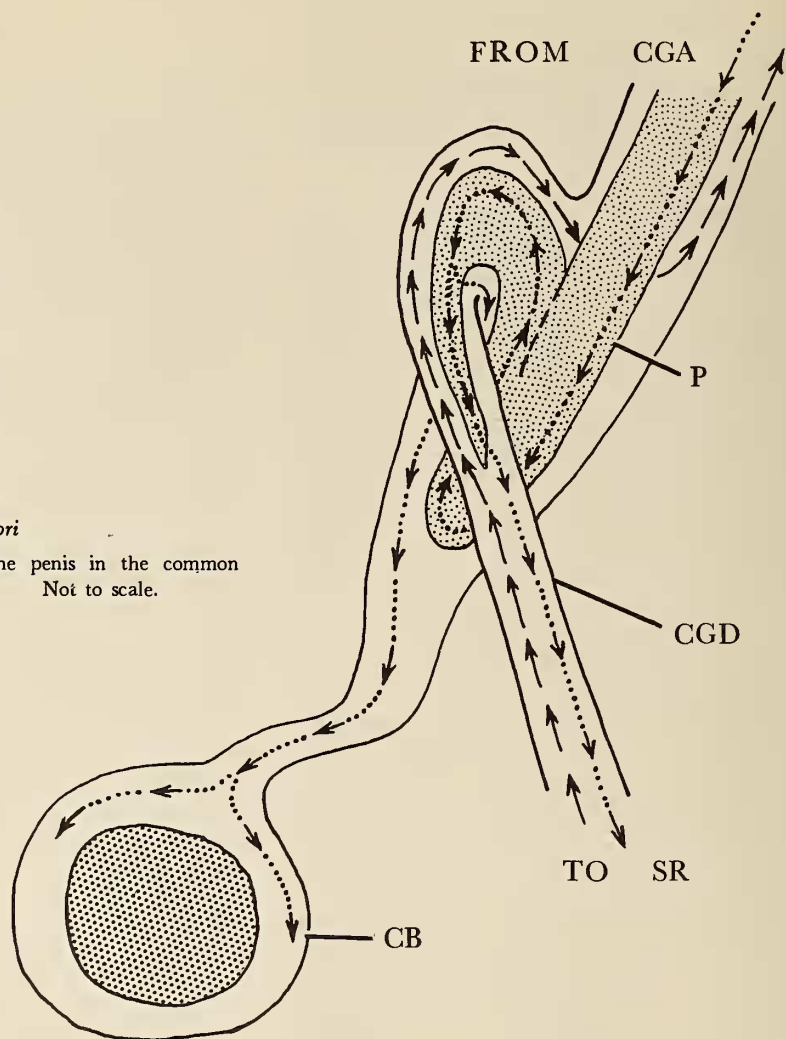
The glans penis is projected as the penis sheath is everted by hydrostatic pressure within the hemocoel. The everted sheath forms a tubular basal section of the functional penis, with the penis retractor muscle extending into and up this tube to its insertion on the base of the glans (Figure 20).

After insertion, the penis is twisted and undergoes pulsations within the copulatory duct until its tip reaches a point just internal to that where the copulatory bursa duct joins the copulatory duct. A loop of the penis may invade the entrance of the copulatory bursa duct, but the tip of the penis bypasses this opening and goes deeper into the copulatory duct (Figures 18, 22, 23; point "T" on Figure 37). The penis is now exceedingly well anchored in the copulatory duct by the following mechanisms: 1) The sphincter of the common genital aperture grasps the penis at its base. 2) The hollow base of the penis has been greatly expanded by hydrostatic pressure to form a bulbous anchor in the cavity of the atrium. Even thus expanded, the walls of the atrium are still much folded and pleated. 3) The penis forms an elbow-like angle within the atrium. This provides both anchorage and a base for the thrust of the glans into the tight, coiled section of the copulatory duct between the atrium and the copulatory bursa duct (Figures 4 and 22). 4) The penial spines are pressed into the female tract by muscular and hydrostatic action (Figure 20). 5) Points within the female tract are pressed against the penis. 6) The very powerful atrium basal muscle (Figure 4) clamps the base of the glans on the inward side of the atrium. Lesser muscular contraction is also evident in the coiled section of the common genital duct. 7) The complex configurations of the coiled section of the copulatory duct provide the final anchorage for the penis (Figure 22).

Figure 18

*Phyllaplysia taylori*

Diagram showing the placement of the penis in the common genital duct during copulation. Not to scale.



### Plate Explanation

Figure 19: The penis and opened penial sheath of *Phyllaplysia taylori*. The genital groove is visible on the inner surface of the sheath. Several penial spines are evident near the junction of the sheath and the coiled glans. From life. Scale line represents  $250\mu$ .

Figure 20: Cross-section of the penis of *Phyllaplysia taylori* killed during copulation. Mayer's haemalum and triosin. Scale line represents  $100\mu$ .

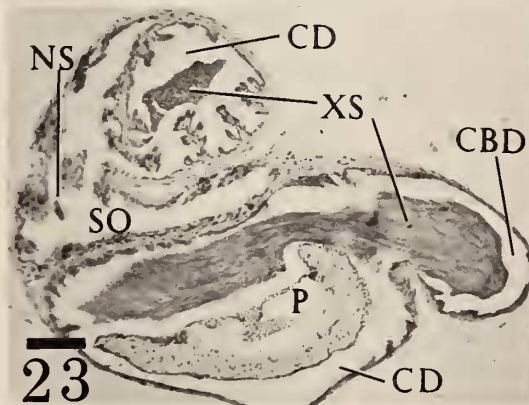
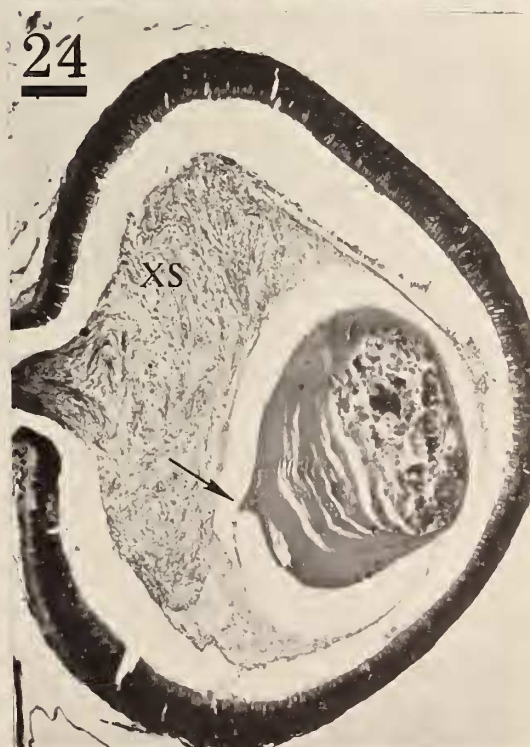
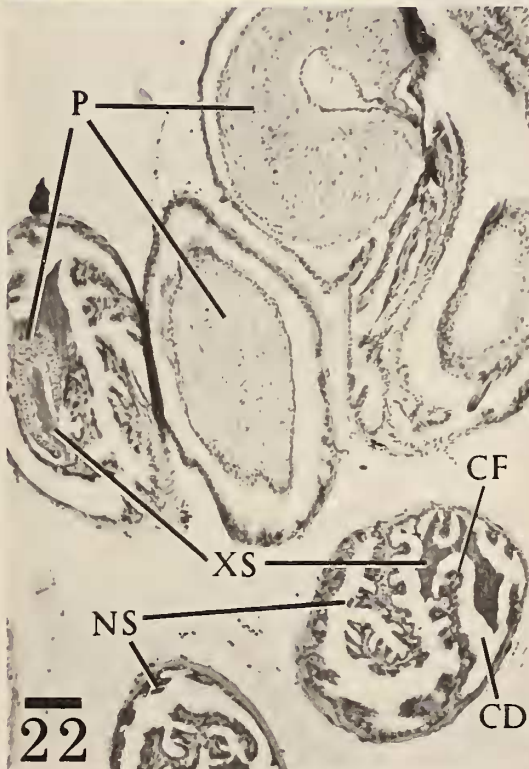
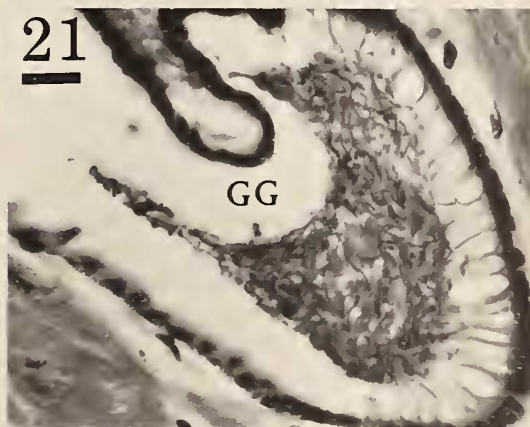
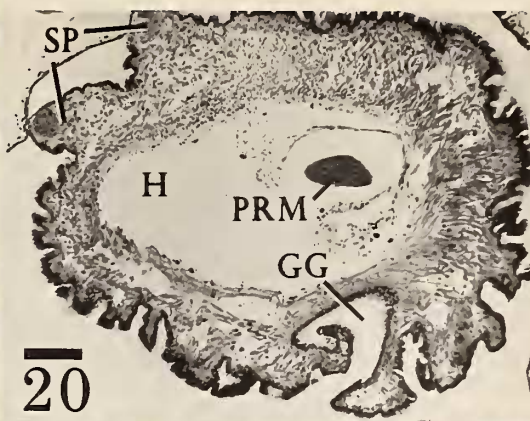
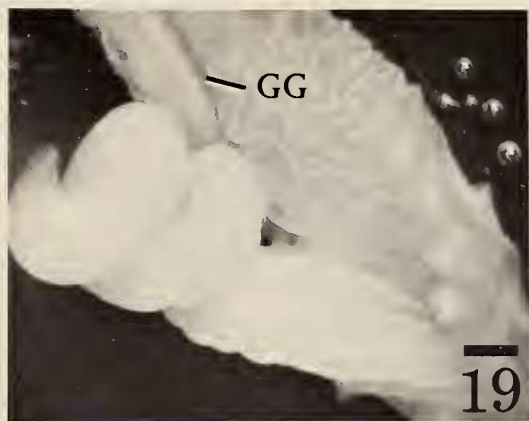
Figure 21: Enlarged view of the genital groove of the above specimen, showing ciliary transport of the seminal mass. Scale line represents  $12\mu$ .

Figure 22: Section of *Phyllaplysia taylori* killed during copulation. All the coils of the glans penis are contained within the tightly spiraled copulatory duct; a deeper continuation of this is marked. Exogenous sperm is being poured into this animal, while the endo-

genous sperm is moving out as a seminal thread. Haemalum and triosin. Scale line represents  $75\mu$ .

Figure 23: A section of the common genital duct, at the level of the copulatory bursa duct, of a *Phyllaplysia taylori* killed during copulation. Exogenous sperm was moving from the penis tip both laterally into the copulatory bursa duct and downward in the copulatory duct toward the seminal receptacle. Endogenous sperm is moving as a seminal strand up the spermiduct. Mayer's haemalum and triosin. Scale line represents  $90\mu$ .

Figure 24: Exogenous sperm pouring into the copulatory bursa of the specimen of Figure 23. The arrow indicates a "tail" which marks the point where semen, probably from a former copulation, stopped flowing into the bursa. Note the yolk granules within the older, darker, contained mass. Mayer's haemalum and triosin. Scale line represents  $80\mu$ .





The lumen of the atrial gland is filled with a weakly staining secretion during copulation. This secretion can be seen to extend out of the atrial gland and surround the passing penis. After copulation the vacuoles of the atrial gland cells appear to be discharged and the entire gland has shrunk.

The penis continues to pulsate during copulation. This may help to keep the penis anchored or it may help in the transport of sperm, either by directly forcing the seminal fluid or by keeping its channel clear despite its many convolutions and compressions.

Copulation is a very extended process. It usually lasts 2 or 3 hours but often continues for more than 4 hours. It is usually a continuous process, but I have seen partners mate for a period, separate, wander about, and then rejoin. The end of copulation is often not a synchronous process. One may withdraw and move about, dragging the still connected partner around for up to 30 minutes.

The long copulations and the elaborate mechanisms for maintaining copulatory contact are probably necessary considering the slow transport of sperm cells by cilia. It doesn't matter if part of the propulsion is later seen to be muscular, for there are at least sections of the tract where a thin seminal strand is moved only by ciliary means, and the speed at these points determines the upper limit of the transfer rate.

The purpose of copulation, of course, is the transfer of semen into a mate. This was typically evidenced by the decrease in size of the ampulla in both partners, clearly visible through their ventral body walls. However, reciprocal copulations were observed in which one partner had an empty ampulla when copulation commenced. Post-copulation dissection revealed that such a partner passed no semen to its mate. Not infrequently other copulations were discontinued when one partner had transferred his semen, while the mate had not yet finished.

The functional morphology of copulation in an aspidi-dean has not previously been described in detail. However, one aspect, of considerable functional import, has been the subject of dispute. This concerns the final placement of the penis tip. MAZZARELLI (1891) believed that in *Aplysia punctata* the tip was diverted toward the copulatory bursa duct by a fold in the copulatory duct. EALES (1921), working on the same species, crudely demonstrated that the penis penetrated deeper, towards the seminal receptacle, by dissecting out a penis which had been severed at its base during copulation. What degree of artifact was present in her experiment is debatable; the penis is not as well anchored in most *Aplysia* as in *Phyllaplysia* and the severe stimulus of severing may have caused unknown changes in penis position or length. LLOYD (1952) found that such severing even caused the

ejection of the inserted penis in the nudibranch *Archidoris*. The present observations on *P. taylori* consistently showed the penis at the position shown in Figures 18, 22, 23, and 37, regardless of the method of fixation or killing, so long as it was very rapid. THOMPSON & BEBBINGTON (1969) reported ejection problems when copulating *A. fasciata* or *A. punctata* were plunged into boiling Bouin's fluid, but they indicate a similar penis penetration for *A. depilans*.

It seems clear that the atrial gland is involved with the lubrication of the penis; it could be considered analogous to the vestibular glands of the human female. The "bursa seminalis" is an organ at the base of the copulatory bursa duct in *Aplysia punctata* which is obviously homologous to the atrial gland of *Phyllaplysia taylori*. The location of this "bursa seminalis" led EALES (1921) to deduce that its function was the agglutination of seminal debris which was then drawn up into the copulatory bursa. The different position of the atrial gland in *Phyllaplysia*, at a point removed from direct contact with exogenous semen, would argue against this. LLOYD (1952, p. 93) suggested that it may contribute prostatic secretion to the outgoing endogenous sperm in *A. punctata*, perhaps even capacitating them. This followed from her erroneous assumption that the endogenous sperm must travel past this organ on the "right side" (copulatory duct) "if they are to avoid the female accessory glands." THOMPSON & BEBBINGTON (1969) also reported that prostatic secretions from similar glands are added to outgoing endogenous sperm.

The atrium is seen to be a relatively passive female structure involved in the anchoring of the hydrostatic bulb of the penis. A similar but much more distinct structure in *Akera* is designated as a "réservoir séminal" by GUIART (1901), and another in *Dolabella* is called the "seminal receptacle" by MACFARLAND (1918). These unfortunate terms are the result of giving anatomical structures functional names before their function has actually been determined.

#### MOVEMENT AND STORAGE OF EXOGENOUS SPERM

The distinctness of the seminal strand is lost as the semen is moved into a mate during copulation. The dissolution of the incoming seminal strand may be due to the distance it has traveled without "prostatic" action, to the pulsating of the penis during copulation, to substances produced within the penis, or to substances present in the copulatory duct. Three observations argue against the last-named

suggestion. First, the strand appears to be loosening even before it is discharged from the penis. Second, accidental tangles of endogenous seminal strands in the atrial area during copulation are not broken up until after copulation. Third, the normal endogenous seminal strand of the animal receiving exogenous sperm is not affected, though it passes through the same area. However, it is well protected from the copulatory duct by folds and perhaps by a mucus seal.

Whatever the reason for breakup of the incoming seminal strand, the breakup itself seems to aid in the movement of sperm from the discharge area. Weak muscular waves move down the common genital duct from the common genital aperture towards the seminal receptacle. These contractions evidently supplement the action of the rather moderately developed cilia of the copulatory duct in moving semen inward. It is interesting to remember that, in the same tube, less than a millimeter away, an endogenous seminal strand is moving in the opposite direction. As previously noted, this outgoing thread is moved by powerful cilia, in an anatomical arrangement which is unaffected by the inward contraction waves.

An abundance of semen is passed into a mate during a typical copulation. Near the end of, and immediately after, copulation in *Phyllaplysia taylori*, exogenous semen fills the copulatory duct below the atrium (Figure 23). This seminal mass has started into the copulatory bursa and the seminal receptacle (Figures 23, 24, 26, and 37). This has been confirmed by vivisections, dissections, and autoradiography. A few mated animals did not have sperm in the copulatory bursa. The sperm are completely unoriented in either seminal receptacle or copulatory bursa at this time.

As noted earlier (BEEMAN, 1970c), labeled exogenous sperm are seen to get barely around the tip of the copulatory fold, and are completely absent from the spermoviduct of unlabeled animals (Figure 22).

Stained sections of the exogenous semen in the copulatory duct reveal many oval to pointed-oval nuclei without distinct cell boundaries scattered among the elongated sperm heads. Phase-contrast preparations suggest that these objects are amebocytes.

The bulk of the exogenous semen in the copulatory duct is moved into the recipient's seminal receptacle within 2 hours after copulation. A few sperm are left stuck in a viscous material which now lines the duct. A few of these sperm are coiled apparently in the epithelium of the duct walls; it is possible that they are being phagocytized. Within 5 hours after copulation the 10% of the exogenous sperm which have penetrated most deeply into the seminal receptacle have become oriented with their heads buried into the receptacle lining (Figure 27). Sperm orientation was not observed ectad from the middle of the seminal receptacle. Several hours after copulation, large, irregularly shaped, clear areas, containing no sperm, were present in the center and upper end, but never the bottom, of the seminal receptacle (Figure 27). The exogenous sperm are maintained in the oriented position in the seminal receptacle until oviposition.

The exogenous semen, if any, which was deposited in the copulatory bursa is left stranded there. It is then compacted, and perhaps digested; no sign of phagocytosis by the walls of the copulatory bursa wall was observed. Examination of Figure 24 shows at least 2 almost concentric sperm masses, an outer one composed of fresh exogenous sperm just added by copulation, and an older inner one which has been compacted and partially destroyed. The new mass has pushed the old one in and surrounded it; the area on the old mass which was formed in the copulatory bursa duct persists as a small "tail." The origin of the older mass is not definitely known. The simplest assumption is that it represents overflow of exogenous sperm from a former copulation, but the presence

### Plate Explanation

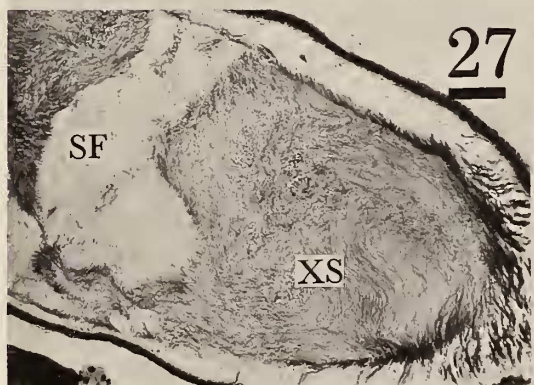
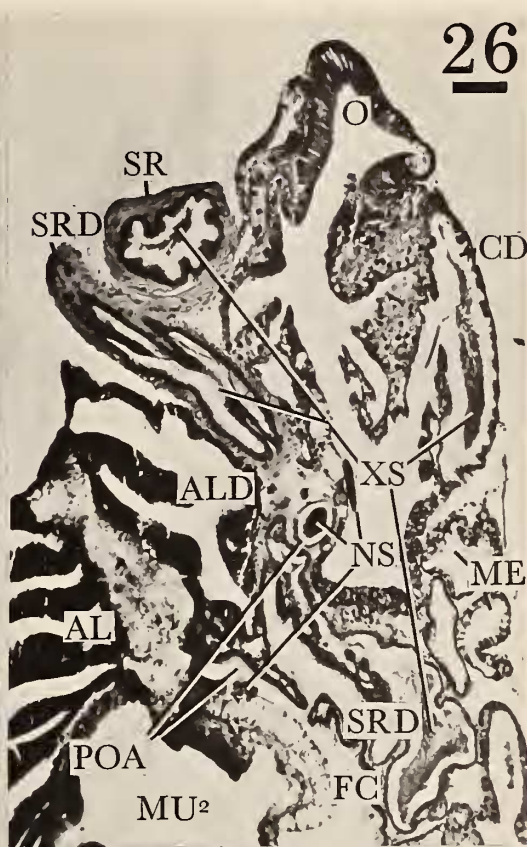
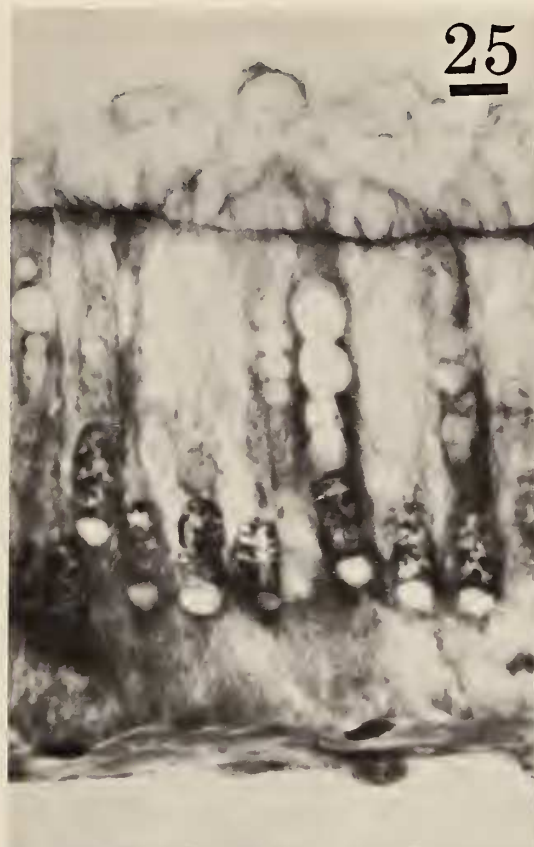
Figure 25: Columnar endothelium of the copulatory bursa wall in *Phyllaplysia taylori*. Mayer's haemalum and triosin. Scale line represents  $60\mu$ .

Figure 26: An angled sagittal section of the female gland mass of a *Phyllaplysia taylori* killed during copulation. Exogenous sperm was moving down the copulatory duct, up the seminal receptacle duct, and into the seminal receptacle. Endogenous sperm was coming up the post-ampullar duct, passing into a loop of the same duct into the fertilization chamber area, and then continuing up the common genital duct as a seminal strand. The oviduct and membrane gland are inactive in this phase. Mayer's haemalum and eosin. Scale line represents  $75\mu$ .

Figure 27: Longitudinal section of a seminal receptacle of a *Phyllaplysia taylori* killed 5 hours after copulation. Note the beginning of orientation of the exogenous sperm and the presence of clear areas of seminal fluid. Scale line represents  $60\mu$ .

Figure 28: A section of the seminal receptacle wall of *Phyllaplysia taylori* killed before exogenous sperm became oriented. Mayer's haemalum and triosin. Scale line represents  $12\mu$ .

Figure 29: As in Figure 28, but now the exogenous sperm have become oriented and attached to the specialized endothelial lining of the seminal receptacle. Scale line represents  $20\mu$ .





of yolk granules in the center of the old mass indicates that at least some endogenous material is present.

As GHISELIN (1964) has remarked, opisthobranch workers have largely overlooked muscular action as a factor in moving materials in the gonoduct. The relation of muscular action to sperm movement discussed above (and to egg string movement to be discussed later) is therefore of special interest. I agree with GHISELIN that speculations, such as those by THOMPSON (1961), LEMCHE (1956), and EALES (1921), invoking the movement of opisthobranch sperm over relatively long distances by means of their own motility, are questionable. The sperm of *Phyllaplysia taylori* are certainly motile; those from the ovotestis, ampulla, seminal receptacle, and to a lesser extent the copulatory bursa, exhibit strong lashing and twisting movements. It is very likely that these are functionally involved in short-range movements such as orientation and fertilization, but the presence of the muscular movements and ciliated tracts already described make it unnecessary to postulate directed swimming movements of sperm over greater distances in the reproductive system.

A number of general discussions of sperm reception must be considered. MAZZARELLI (1891) believed that the sperm in *Aplysia* are ejaculated and forced to flow entirely into the copulatory bursa. He stated that exogenous sperm leave the seminal material in the bursa and then pass down the copulatory duct to accumulate in the seminal receptacle. EALES (1921) felt that the seminal discharge and purification both occur in the copulatory duct of *A. punctata*. She suggested the sperm then move to the seminal receptacle and the debris is swept into the copulatory bursa. Her modification of MAZZARELLI's scheme is schematically diagrammed in Figure 32. EALES' conclusions have served as a model for most later discussions of anaspidean reproductive function. McCauley's (1960) discussion and drawing of the reproductive system of *Phyllaplysia taylori* agree with her model almost completely. However, his morphological study included only side references to the reproductive functions; the discussion and diagram are therefore very incomplete on this point.

MARCUS (1961a) has published the only diagram of the reproductive system of *Phyllaplysia taylori* in which the routes for gametes can be traced (see the anatomy section for my many objections to the anatomical relationships indicated by this diagram). MARCUS did not discuss reproductive function in *P. taylori*. I have attempted to illustrate MARCUS' ideas in a schematic diagram (Figure 34); this presents his concept of *P. taylori*'s anatomy and incorporates his comments (MARCUS & MARCUS, 1957a) on reproductive function in *Aplysia cervina* insofar as these are consistent with his diagram of *P. taylori*.

It must be emphasized that MARCUS might have shown these matters quite differently had he actually worked on reproductive function in *P. taylori*. Although lacking a definitive way to distinguish between endogenous and exogenous sperm, THOMPSON & BEBBINGTON (1969) report sperm routings in 3 species of *Aplysia*, which are similar to the radiolabeling results in *P. taylori*.

Many of the main points of agreement and difference between my work and that of others can be most easily seen by comparing the schematic diagrams of Figures 32, 33, 34, and 37. The copulatory bursa and seminal receptacle require special discussion which is deferred until the movement and deposition of ova has been discussed.

## EGG-STRING PRODUCTION AND MOVEMENT

### 1. Movement of Oocytes

Several hours to days normally elapse between copulation and oviposition in *Phyllaplysia taylori*. Preparatory to oviposition groups of oocytes accumulate in the lumen of the ovotestis lobes as they are shed from the ovotestis walls. They move, evidently by ciliary action, singly into the dendritic channels of the pre-ampullar duct being propelled along the ciliary band of the ampulla and then neatly bypass masses of sperm stored in the ampulla. This ciliary propulsion gradually results in oocytes filling the anterior region of the ampulla.

Egg-string production begins with the ciliary movement, perhaps with some muscular assistance, of the loose oocytes stored in the ampulla into a more or less single-file train up the post-ampullar duct. This passage is so narrow that only the single-file arrangement of the still unconnected oocytes is possible at most points, but several can be seen at one time in the small bulge of this duct. The post-ampullar duct arches over the albumen gland and slowly delivers the oocytes by ciliary action to the fertilization chamber. Development of the oocytes up to this point has been halted at metaphase of the first meiotic maturation division.

### 2. Fertilization

Within 3 hours after copulation the seminal receptacle is packed and distended with sperm; the more distal material is brownish. As ciliary action passes the loose, single oocytes through the fertilization chamber, the entire seminal receptacle exhibits vigorous constriction and dilation. Peristaltic waves start at the base of the receptacle neck and push sperm down the duct and into the fertilization chamber. This wave triggers a reverse wave which

then quickly and progressively closes the lumen of the duct. This process is repeated every few seconds. Oocytes and exogenous sperm are mixed as the oocytes continue in a loose association through the fertilization chamber.

### 3. Egg-String Formation and Oviposition

The routing of the egg-string in the female gland mass can best be followed by considering the following discussion in conjunction with Figures 35, 36, and 37.

As fertilization proceeds, the albumen gland discharges its secretion into the fertilization chamber by an undetermined mechanism. An irregular line of eggs covered with a loose mush of albumen results and is slowly moved into the membrane gland by ciliary action. The limited opening of the membrane gland forces the ova (which are still independent of each other) to again become almost single-file. An indistinct strand of eggs, heavy with their loose albumen coverings, then starts moving by ciliary action through the complex sacculations of the membrane gland. The first sacculations start to smooth the albumen coats of the ova. The strand is too large to enter the tiny groove which follows the distal margins of these flattened sacculations, but its movement seems to be aided by the well-developed cilia present there. Soon after the strand has entered the first portion of the membrane gland (the region which stains darkly with methyl green) the strand begins to look more compact and to glisten. A distinct and coherent egg-string is gradually developing, covered with a membrane-like film. The egg-string is slowly rotated by ciliary action as it moves along the oviducal route; each egg, within a flattened ovoid of albumen, is individually wrapped in the membrane, and the membrane between adjacent ova becomes spirally twisted. This mechanism of wrapping the eggs supports the observations of GHISELIN (1965) who, THOMPSON & BEBBINGTON (1969) claimed, was in error. The egg-string continues to move through the spiraled and reverse-spiraled sacculations, into the final and apparently non-glandular section of the membrane gland. From here the egg-string continues along the increasingly more distinct oviducal groove into the primary lobe of the mucous gland, still moved by ciliary action alone. The string compresses slightly as it enters the mucous gland so that for a short span it is 2 or 3 eggs across and thus flattened, and about 3 times as wide as thick.

The egg-string is now moved along the double U-shaped oviducal groove, which marks the edge of the mucous gland, first along the small U of the primary lobe, then straight through the female gland mass to the large U of the secondary lobe. Mucus, secreted by the large, non-ciliated, secretory part of this gland, moves peripherally to the oviducal groove and is added in layers to the rotating egg-string. The egg-string thus gains diameter as it passes and shortly after entering the mucous gland it becomes round in cross-section again. Secretory cells found directly in the oviducal groove perhaps serve to lubricate the string's passage.

The finished egg-string contains eggs which are quite uniform; they have a diameter of about  $110\mu$  ( $100\mu$  to  $116\mu$ ) in fixed sections, and a nucleus of about  $25\mu$  across. The addition of the membrane and albumen had increased the diameter of the egg-string to about  $220\mu$  ( $200\mu$  to  $250\mu$ ) and the addition of mucus brings the total diameter to about  $540\mu$  ( $515\mu$  to  $610\mu$ ).

A mucus seal between the tip of the spermoviduct and the axial, weakly ciliated side of the egg guide turns most of the oviducal groove of the spermoviduct into a true tube during egg passage (Figure 17). Within this tube the egg-string is carried forward by powerful cilia. The secretory cells lining the oviducal groove are obviously highly active during this phase. Several functions, or combinations of functions, are possible for this secretion. Suggestions include egg-string lubrication (MAZZARELLI, 1891) and treatment of the egg-string with bacteriostatic materials (C. B. vanNeil, pers. comm., 1966). FRETTER & GRAHAM (1962) report that similar glands in proso-branches aid ova in sticking to the substrate. Before the spermoviduct gland is referred to as a "prostate" it should be noted that it is here more involved with the egg-string than with the semen.

The spermoviduct narrows into an internal seminal groove at the level of the copulatory bursa duct. The seminal groove, which prior to copulation carries the endogenous seminal thread, spirals around the wall of the common genital duct above this point. The egg-string, in contrast, travels directly through the cavity around which the internal seminal groove coils; it simply follows the shortest possible duct to the common genital aperture. The cavity along which the egg-string travels also serves as the outer part of the copulatory duct. Simultaneous copulation and egg-laying have not been observed in

### Plate Explanation

Figure 30: Scanning electron micrograph of the lumen surface in the seminal receptacle of *Aplysia californica*. Oriented bundles of sperm filaments project upward into the lumen. The anterior ends of these exogenous sperm spread over, between, and into the rounded heads of the endothelial cells. Scale line represents  $5\mu$ .  $20^\circ$  beam angle, 20 kv.

Figure 31: Scanning electron micrograph similar to Figure 30, but at higher magnification. The luminal end of an endothelial cell, with exposed microvilli and associated secretions, fills the center of this micrograph. The anterior end of the spermatozoon marked by the arrow is buried into this endothelial cell. Scale line represents  $1\mu$ .  $20^\circ$  beam angle, 30 kv.

